

ADOPTED: 24 May 2017

doi: 10.2903/j.efsa.2017.4873

Guidance on dermal absorption

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Abstract

This guidance on the assessment of dermal absorption has been developed to assist notifiers, users of test facilities and Member State authorities on critical aspects related to the setting of dermal absorption values to be used in risk assessments of active substances in Plant Protection Products (PPPs). It is based on the 'scientific opinion on the science behind the revision of the guidance document on dermal absorption' issued in 2011 by the EFSA Panel on Plant Protection Products and their Residues (PPR). The guidance refers to the EFSA PPR opinion in many instances. In addition, the first version of this guidance, issued in 2012 by the EFSA PPR Panel, has been revised in 2017 on the basis of new available data on human *in vitro* dermal absorption for PPPs and wherever clarifications were needed. Basic details of experimental design, available in the respective test guidelines and accompanying guidance for the conduct of studies, have not been addressed but recommendations specific to performing and interpreting dermal absorption studies with PPPs are given. Issues discussed include a brief description of the skin and its properties affecting dermal absorption. To facilitate use of the guidance, flow charts are included. Guidance is also provided, for example, when there are no data on dermal absorption for the product under evaluation. Elements for a tiered approach are presented including use of default values, data on closely related products, *in vitro* studies with human skin (regarded to provide the best estimate), data from experimental animals (rats) *in vitro* and *in vivo*, and the so called 'triple pack' approach. Various elements of study design and reporting that reduce experimental variation and aid consistent interpretation are presented. A proposal for reporting data for assessment reports is also provided. The issue of nanoparticles in PPPs is not addressed. Data from volunteer studies have not been discussed since their use is not allowed in EU for risk assessment of PPPs.

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Keywords: dermal absorption, plant protection products, *in vitro*, *in vivo*, triple pack, default values

Requestor: European Commission

Question number: EFSA-Q-2015-00633

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Update: This scientific output, approved on 24 May 2017, supersedes the previous output published on 25 April 2012 (EFSA PPR Panel, 2012). The European Commission will decide the implementation time for the mandatory use of this guidance in the regulatory context.

Acknowledgements: EFSA wishes to thank Olavi Pelkonen and the other members of the Panel on Plant Protection Products and their Residues for the scientific review and endorsement, respectively, of this scientific output.

Suggested citation: EFSA (European Food Safety Authority), Buist H, Craig P, Dewhurst I, Hougaard Bennekou S, Kneuer C, Machera K, Pieper C, Court Marques D, Guillot G, Ruffo F and Chiusolo A, 2017. Guidance on dermal absorption. *EFSA Journal* 2017;15(6):4873, 60 pp. <https://doi.org/10.2903/j.efsa.2017.4873>

ISSN: 1831-4732

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Summary

The European Food Safety Authority (EFSA) was asked by the European Commission to assess the scientific quality of newly available studies on human *in vitro* dermal absorption and, based on the evaluation of the studies, to revise the EFSA PPR Panel guidance on dermal absorption (EFSA PPR Panel, 2012).

Data on human *in vitro* dermal absorption for Plant Protection Products (PPPs) were made available from the European Crop Protection Association (ECPA) and from the Bundesinstitut für Risikobewertung (BfR) by submitting the study reports and two data sets with extracted information from the *in vitro* experiments. A quality check was performed in order to verify if new studies were conducted according to the current regulatory standards and to identify potential deviations from the EFSA PPR Panel guidance on dermal absorption. In addition, a plausibility check to validate the correctness of data entry in the data sets from sources (study reports) was undertaken applying a two tiers (I and II) validation protocol. The evaluation of the scientific quality of the new studies described in the EFSA scientific report 'Assessment of new scientific studies on human *in vitro* dermal absorption' (EFSA, 2015) indicated that, although a number of deviations from the application of the EFSA PPR Panel guidance on dermal absorption have been identified, the new studies comply with the current regulatory standards. In the report, it was recommended to analyse the dermal absorption data applying a model-based statistical protocol and revise the EFSA PPR Panel guidance on dermal absorption issued in 2012 accordingly (EFSA PPR Panel, 2012).

An updated version of the data sets including individual values was subsequently submitted by ECPA and BfR. In addition to a two-tiered quality check of submitted study reports (EFSA, 2015), a plausibility check to verify the correctness of the information available in the updated data sets from the sources (study reports) confirmed a high quality of data entry. Data analysis provided the scientific basis for the revision of the guidance on dermal absorption.

In order to set new default values to be used in the absence of experimental data, two different statistical modelling approaches are applied to the combined ECPA and BfR data set. In addition, empirical percentiles are calculated and proposed values for setting default values with quantified uncertainties are assessed. Advantages and disadvantages of both empirical and model-based approaches are also evaluated.

The statistical analysis indicates a significant impact on dermal absorption of concentration status ('concentrate' or 'dilution') and formulation category, thus new default values for concentrated pesticide formulations categorised by formulation type and their dilutions are set in the revised guidance.

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1. Introduction

This guidance is designed to assist notifiers, test facilities and Member States' Authorities on critical aspects related to the setting of dermal absorption values to be used in risk assessments of active substances in plant protection products (PPPs) reviewed for approval under Regulation (EC) No 1107/2009¹ and Regulation (EU) No 284/2013².

The document is aimed at providing guidance based on the available science in order to improve consistency of data derivation, presentation and interpretation. Where data are equivocal or lacking, existing practises and/or recommendations in other regulations/guidance documents are proposed to be followed since overall, taking into account the uncertainties involved, it is the EFSA PPR Panel's opinion that the relevant dermal absorption estimates will be sufficiently protective in these cases [see the scientific opinion on the science behind the revision of the guidance document on dermal absorption issued by the EFSA Panel on Plant Protection Products and their Residues (PPR) in 2011].

This document does not address every possible scenario and it is expected that case-by-case judgement will be needed in some instances. Where case-by-case assessments are necessary, they should be designed to provide the same level of scientific rigour as an assessment performed using the standard principles described in the guidance document, and the evidence and reasoning involved should be fully documented.

Internationally agreed guidelines exist for the performance of dermal absorption studies *in vivo* and *in vitro* (OECD, 2004a,b; Regulation (EC) No 440/2008³). These test guidelines are designed to cover all types of chemicals and dermal exposure scenarios, not just pesticide formulations. Notably, they give only minimal guidance on the interpretation of results. This document does not address basic details of experimental design, which are addressed in the EC test methods B.44 and B.45 [Regulation (EC) No 440/2008] and in the related OECD test guidelines 427 and 428 (OECD, 2004a,b) and supporting guidance (OECD, 2004c). It does, however, provide recommendations for performing and interpreting dermal absorption studies with PPPs in order to reduce variability among studies and to improve data reliability and interpretation. The potential applicability of this guidance to exposures to other chemical classes (e.g. biocides or industrial chemicals) will need to be determined by the bodies responsible for such evaluations.

This guidance also covers scenarios where there are no data on dermal absorption for the product under evaluation and different types of data are available for either the product under evaluation or related products or the active substance itself. Flow charts were considered an important part of the guidance and are therefore included.

The issue of nanoformulations in PPPs is not addressed. Currently, there is insufficient information on the penetration of nanoparticles through the skin. It is considered that, at present, evaluation of all aspects of nanoparticle-based PPPs should be performed on a case-by-case basis (WHO, 2006).

In the EFSA PPR Panel 'Opinion on the science behind the revision of the guidance document on dermal absorption' more detailed explanations and rationales for the decision tools presented in the guidance are provided (EFSA PPR Panel, 2011). Every effort has been made to accurately cross-reference the relevant sections of the opinion.

Moreover, the EFSA PPR Panel guidance issued in 2012 has been revised in 2017 by the EFSA Working Group on Dermal Absorption, on the basis of newly available data on human *in vitro* dermal absorption for PPPs and wherever further clarifications were needed. Accordingly, Background and Terms of References have been updated.

¹ Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EC and 91/414/EEC. OJ L 309, 24.11.2009, p. 1–50.

² Regulation (EU) No 284/2013 of 1 March 2013 setting out the data requirements for plant protection products, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. OJ L 93, 3.4.2013, p. 85–152.

³ Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). OJ L 142, 31.5.2008, p. 1–739.

1.1. Background and Terms of Reference as provided by EFSA

1.1.1. Background as provided by EFSA

The preparation and revision of the EU Guidance Documents to assist the implementation of Council Directive 91/414/EEC was originally the responsibility of the European Commission; this remit has been transferred to the European Food Safety Authority (EFSA) regarding risk assessment Guidance Documents. The need for update/revision of the Guidance Document on Dermal Absorption (SANCO/222/2000 rev.7, 19 March 2004) was expressed by Member States, consulted in 2006. This message was reinforced in the report from the 2009 public consultation carried out by EFSA on the Guidance Document, and it was clearly indicated that there was a need for substantial changes.⁴

In an EFSA outsourced project published in 2010, these comments as well as available databases on dermal absorption were analysed and relevant literature was reviewed. In the report thereof, further needs for updates and recommendations for a revised Guidance Document were presented.⁵ Moreover, the EFSA PPR Panel developed an 'Opinion on the Science behind the Revision of the Guidance Document on Dermal Absorption' (EFSA PPR Panel, 2011), providing relevant data, evaluations and references that supported the criteria to facilitate assessment of dermal absorption of active substances in PPPs under Regulation (EC) No 1107/2009.

On this basis, the EFSA PPR Panel was asked by the European Commission to prepare a revision of the Guidance Document on Dermal Absorption. During the development of the guidance, the EFSA PPR Panel had a consultation with the Standing Committee on the Food Chain and Animal Health (SCoFAH) on risk management issues of the guidance. Among the issues considered were the default values to be used when data is lacking and the value of ± 25 w/w content of types of co-formulants for read across between different formulations. The proposed values were confirmed. Subsequently, the SCoFAH took note of the Guidance Document and it entered into force in the European Union (EU) by December 2012.

In April 2013, the European Crop Protection Association (ECPA) indicated to the European Commission and to EFSA that the guidance was, in their opinion, excessively conservative and that it did not take into account new relevant scientific studies. In particular, ECPA referred to a manuscript submitted to a scientific journal for publication in which several amendments to the EFSA PPR Panel guidance on dermal absorption were proposed (Aggarwal et al., 2014).

EFSA accepted to consider the new data, provided that access to the original study reports referred to in the scientific publication was ensured. Agreement was reached with ECPA that confidentiality about the original study reports would be respected within the limits of Regulation (EC) No 1049/2001⁶ on EU rules for access to documents. The study reports were submitted to EFSA for assessment in June 2014.

In September 2014, ECPA indicated that an additional 170 *in vitro* dermal absorption studies with human skin conducted during the period 2012–2014 had been added to the existing database of approximately 190 studies conducted until 2012. The additional studies were submitted to EFSA in October 2014, together with a draft manuscript (Aggarwal et al., 2015).

After consultation of Member States by the European Commission, Germany informed EFSA that a structured database containing a large data set on human *in vitro* dermal absorption had been compiled by Bundesinstitut für Risikobewertung (BfR). Data have been extracted from original study reports of PPP submissions and the database includes also rat *in vivo/in vitro* dermal absorption data.

1.1.2. Terms of Reference as provided by EFSA

The European Commission asks EFSA:

- 1) To assess in the first instance the scientific quality of all the new studies available and to compile a comprehensive database of the dermal absorption studies. This should include all studies in line with the regulatory standards for this type of experiment and made available by the industry as well as by public institutions.

⁴ <http://www.efsa.europa.eu/en/scdocs/scdoc/282r.htm>

⁵ <http://www.efsa.europa.eu/en/scdocs/scdoc/52e.htm>

⁶ Regulation (EC) No 1049/2001 on Access to European Parliament, Council and Commission documents. OJ L 145, 31.5.2001, p. 43–48.

- 2) Subsequently, based on the evaluation of the studies, to consider whether the current guidance on dermal absorption shall be revised or, if more appropriate, a new guidance developed.

The evaluation of scientific quality of new studies described in the EFSA scientific report 'Assessment of new scientific studies on human *in vitro* dermal absorption' (EFSA, 2015) indicated that, although a number of deviations from the application of the EFSA PPR Panel guidance on dermal absorption issued in 2012 have been identified, the new studies comply with the current regulatory standards. EFSA's recommendations to address the variability within studies and to improve the consistency of interpretation have not been applied routinely. In addition, the data sets as provided to EFSA were not completed, lacking individual data or other information addressing variability. However, the overall conclusion regarding the scientific quality of the provided data sets is that they are uniform and solid, and include a relevant number of *in vitro* dermal absorption studies with human skin. In the report, it was thus recommended to analyse dermal absorption data applying a model-based statistical protocol and to revise the EFSA PPR Panel guidance on dermal absorption issued in 2012.

An updated version of the data sets including individual values was subsequently submitted by ECPA and BfR. The quality check to verify the correctness of the information available in the updated data sets from the sources (study reports) confirmed a high quality of data entry. Data analysis provided the scientific basis for the revision of the guidance on dermal absorption

A public consultation and Pesticides Steering Network consultation on the draft guidance on Dermal Absorption was conducted from 22 December 2016 to 24 February 2017, in order to collect feedback from stakeholders and provide needed changes to the text, before PPR Panel endorsement, formal approval by EFSA and publication on the EFSA website.

1.1.3. Interpretation of the Terms of Reference

The EFSA Working Group on Dermal Absorption is asked to prepare a revision of the EFSA PPR Panel guidance on dermal absorption issued in 2012 on the basis of the evaluation of new human *in vitro* dermal absorption studies submitted by ECPA and BfR (EFSA PPR Panel, 2012). Since only *in vitro* data on human skin were supplied, considerations involving *in vivo* data or data on other animal species cannot be re-evaluated. However, the following data-based considerations present in the current guidance can be and are reconsidered based on the newly provided data:

- The number of replicates/donors in the experiment and variations (Section 5.3).
- How to define outliers (Section 5.3)?
- Default values for concentrated and diluted products, based on formulations (Section 6.1).

Both experimental and data interpretation aspects of *in vitro* dermal absorption described in the guidance will be updated when the evidence from new data indicates the need for more clarity, such as for:

- t0.5 calculation (Section 5.1);
- recovery (Section 5.2);
- pro-rata corrections for untested dilutions (Section 5.5);
- exclusion of tape strips (Sections 5.6 and 5.8⁷).

The approach for rounding of values (Section 5.4) will be revised. The section on the use of data on similar formulations (Section 6.2) will be updated to be in line with regulatory requirements and to include indications for formulations containing more than one active substance. Whenever needed, based on changes to the guidance reported above, general Sections 2 and 4, and flow charts (Section 8) will be updated (e.g. new flow chart 1a for selecting default absorption values, flow charts 3 and 6). Moreover, the evaluation of possible inconsistencies/deviations among different guidance/guideline documents on dermal absorption will be conducted to identify possible needs for harmonisation.

Lastly, considerations on the use of quantitative structure–activity relationships (QSAR) will be updated to reflect recent development in the field (Section 6.5.1).

Changes based on the evaluation of new data/information are described under the following sections:

⁷ For consistency, respective clarifications were included in the *in vivo* sections.

- 1) Appendix A: Human *in vitro* dermal absorption data sets: new versions (ECPA and BfR).
- 2) Appendix B: Statistical analysis.
- 3) Appendix C: Evaluation of dermal absorption guidance/guideline documents.
- 4) Appendix D: Evaluation of literature on QSAR for skin absorption prediction.

2. The skin and properties affecting dermal absorption

Below is a summary of relevant information that is presented in the PPR Panel opinion (EFSA PPR Panel, 2011) and in more detailed reference texts (e.g. Marzulli and Maibach, 1996; WHO, 2006; Zhai et al., 2008; Dumont et al., 2015).

- The main barrier to absorption of chemicals is the outermost layer of the epidermis, the *stratum corneum*, which is typically made of 15–20 layers of non-viable cells.
- The *stratum corneum* varies in thickness with anatomical site and species (10–600 μm). Hair follicles and sweat and sebaceous gland density can influence dermal absorption.
- Human skin is considered to be less permeable than that of laboratory animals (WHO, 2006; Monteiro-Riviere, 2008; Holmgaard and Nielsen, 2009).
- Different anatomical sites in humans display a hierarchy of absorption: scrotum > forehead > torso and arms > palms and soles of feet (EFSA PPR Panel, 2011, section 2.2).
- Dermal absorption studies normally use the back (*in vivo* studies) or breast/abdomen/back or upper leg (*in vitro* studies) that are considered to provide realistic dermal absorption values for use in exposure modelling (EFSA PPR Panel, 2011, section 2.2).
- Data on the impact of blood flow/vasodilatation are inconsistent (see opinion section 2.3) and are considered non-relevant variables.
- Sweating and skin hydration have been reported to increase dermal absorption < 2-fold (EFSA PPR Panel, 2011, section 2.3) and are not considered relevant variables, also because they are covered by the intra-species variability factors.
- Significant skin irritation is not expected to occur in normal settings and hence to enhance dermal absorption, except when irritants and/or sensitisers are present in the formulation. However, skin irritation by the active substance and/or by the formulation is already taken into account from the respective dermal irritation tests. This would not be the case if a formulation has sensitising potential only (EFSA PPR Panel, 2011, section 2.4).
- The presence of limited areas of damaged skin is not expected to increase the total absorption (EFSA PPR Panel, 2011, section 2.4).
- The higher permeability (up to twofold) of the skin of an atopic individual is adequately covered by the safety factors applied to derive the acceptable operator exposure level (AOEL) (EFSA PPR Panel, 2011, section 2.4).
- Age-dependent differences in skin properties and functions do not require a separate approach for children and adults when determining absorption values (EFSA PPR Panel, 2011, section 2.5).
- Skin metabolism, although much less active than hepatic metabolism, may affect the kinetics of absorption and alter the nature of systemically available chemicals (Dumont et al., 2015). Especially for *in vivo* methods, it is important to underline the need to cover possible metabolites or marker compound (or compounds) if non-radiolabelled compounds are used. However, it is considered that skin metabolism will not alter the total calculated absorption significantly, as the metabolically active cells are below the *stratum corneum* and the main barrier to absorption of pesticidal active substances has been passed. In addition, as the determination of skin absorption for risk assessment considers the total percentage penetration of a compound into and across the skin, metabolism is not a critical factor in interpreting the data (EFSA PPR Panel, 2011, section 3.8).
- Properties of the active substance that affect absorption include (EFSA PPR Panel, 2011, section 2.6):
 - octanol/water partition coefficient ($\log P_{ow}$)
 - molecular size
 - ionisation

- Other factors affecting absorption (EFSA PPR Panel, 2011, section 2.6):
 - solvents
 - surfactants
 - dilution
 - partitioning between solvent and *stratum corneum*
 - particle size (for powders, SC's, etc.)
 - amount of formulation per unit skin area.

Review of available data on pesticide formulations and new human *in vitro* studies indicates that (see EFSA PPR Panel, 2011, section 4 and Appendix B):

- octanol/water partition coefficient of the active substance ($\log P_{ow}$) and molecular weight (MW) alone were not found to be good predictors of absorption of pesticide formulations;
- relative absorption (e.g. expressed as a percentage of the applied dose) is generally inversely related to the concentration of the active substance. Exceptions may include irritant and volatile compounds, and the presence of co-formulants that strongly affect absorption;
- formulation type is an important factor for dermal absorption that should be considered in setting default values for concentrated products and (in use) dilutions to be used in the absence of data;
- pro-rata correction for untested dilutions is a conservative but appropriate approach in the absence of data.

Based on these observations and considering the fact that pesticide formulations contain solvents and surfactants, ideally, dermal absorption data on PPPs should be generated on the formulated product and on concentrations representative of the spray dilutions as applied to the crop, including the lowest concentration of the active substance (greatest spray dilution). In case adjuvants are recommended to be added to the spray dilution, the test item should contain these adjuvants as well due to an expected impact on dermal absorption.

3. Elements for a tiered approach

Keeping in mind that Regulation (EC) No 1107/2009 and Regulation (EU) No 284/2013 foresee the use of default values as a first tier approach in the absence of data, the assessment of dermal absorption of PPPs can be performed in a structured manner using the criteria outlined in Section 4. This will ensure the best use of resources (for notifiers, contract laboratories and regulators), including a significant reduction in the use of animals, and provide the highest level of confidence in the outcome. Before conducting any studies involving experimental animals, exposure assessments should be performed using default values or existing relevant information, including those from *in vitro* studies (see Section 6, flow charts 1a, 1b and 2).

Below is a list of studies and approaches that can be used in the suggested tiered approach (flow charts 1a, 1b and 2), taking into account that scientifically sound human volunteer *in vivo* data, even if ethically performed, cannot be used for PPPs in the EU.⁸

The following list reflects different levels of refinement:

- Default values or data on closely related products can be used in an initial exposure assessment (see Sections 6.1 and 6.2).
- *In vitro* studies using human skin should be performed before testing in animals is considered.
- Existing data on rats (or other experimental animals): It is widely accepted that results from animal models will over-predict human dermal absorption. Therefore, if animal data from more than one well-designed and well-performed study are available (either *in vitro* or *in vivo*), it is justified to use the lower dermal absorption value.
- 'Triple pack' approach: Existing *in vivo* data in rats (or other experimental animals) are corrected for the ratio of absorption between rats and humans *in vitro*. It should be noted that this will not necessarily provide a value lower than the human *in vitro* data alone (see Section 5.9, flow chart 2 and EFSA PPR Panel, 2011, section 5.4). Rat data generally overpredict dermal absorption, and therefore, it is justified to take the lower value from human *in vitro* data in case this occurs.

⁸ Regulation (EC) No 1107/2009.

4. Elements of a study design and reportings that reduce experimental variation and aid consistent interpretation

In order to improve consistency of interpretation of *in vivo* and *in vitro* study data, it is important that the study protocols are standardised. The EC test methods B.44 and B.45 are designed to cover all types of chemicals and therefore provide a high level of flexibility. This flexibility can lead to a wide variation in designs for dermal absorption studies on PPPs and hence variability in how the studies are interpreted. It is therefore proposed that any dermal absorption studies on PPPs are performed according to EC test methods B.44 and B.45, but with additional considerations:

- 1) Tests should use the formulated product being considered for authorisation.
 - (If not, it may be possible to extrapolate between similar formulations – see Section 6.2)
- 2) In addition to the concentrated product, the greatest dilution (lowest concentration) of the product recommended for use should be tested. If a wide range of dilutions is proposed, then more than one dilution should be tested so that the greatest and smallest recommended dilution rates are covered.
 - (If the greatest dilution recommended on the label has not been tested, the pro rata correction can be made from the highest dilution tested; see Section 5.5 for further details.)
- 3) *In vitro* studies with human skin should preferably use split-thickness (200–400 µm) (dermatomed) skin and be from the abdomen, back, breast or upper leg. This is to improve consistency and comparability particularly as split-thickness membranes tend to have significantly lower levels of residual material than full-thickness preparations (OECD, 2004c; WHO, 2006; Wilkinson et al., 2006; see EFSA PPR Panel, 2011, section 3.1). The use of epidermal membranes may, in some cases, overestimate human *in vivo* skin absorption because of insufficient barrier function (see EFSA PPR Panel, 2011, section 3.1). The use of cultured and reconstructed human skin models (e.g. constructed from keratinocytes) is not recommended for the determination of dermal penetration as these models have not been validated for dermal absorption studies and there are reports that their barrier function is not comparable with that of skin of 'natural origin' (WHO, 2006; SCCS, 2010).
 - (If full-thickness skin is used, the main difference from split-thickness skin is in the amount of material in the receptor fluid and the flux, with the sum of receptor fluid plus skin sample being similar for both split-skin and full-thickness samples (Wilkinson et al., 2006; Vallet et al., 2007). Therefore, by including all material remaining in the skin sample, a dermal absorption value can be obtained. However, any calculated fluxes should not be used).
- 4) *In vitro* studies with rat skin should preferably use split-thickness (200–400 µm) skin from the abdomen or back.
 - (If not, see point 3 above.)

The integrity of human or rat skin used *in vitro* should be determined prior to application of the test substance and should be documented. Various methods can be used (e.g. transepidermal resistance, transepidermal water loss or reference substance penetration) (see OECD, 2004c, para. 42–46). Any membrane with unacceptable integrity should be replaced prior to application. Post-dosing evaluation of integrity instead of predosing evaluation, and subsequent exclusion of results obtained with skin having insufficient integrity is not recommended.

- 5) Solid material should be moistened with a minimal volume of vehicle (e.g. water or physiological saline) to make a paste. This is to mimic sweat on the skin or occlusive conditions under clothing. Since dermal exposure to granular products is usually in the form of dust, the granules should be ground and moistened before application to the skin. Organic solvents should not normally be used (see EFSA PPR Panel, 2011, section 3.2.)
 - (If solids are not moistened then the validity of the study is questionable. If solids are not moistened but occlusive conditions are used then the study can be considered a reasonable match to actual exposures, except for granules.)

- 6) After tape stripping, strips should be analysed separately to permit a profile of the residual material to be determined (for further details see Section 5.1 and EFSA PPR Panel, 2011, section 3.6). Glued (e.g. cyanoacrylate superglue) tape strips should not be used.
 - (If tape strips are not reported individually, then it is not possible to determine whether or not the residue is at the surface or in the lower layers. In such cases, the tape strips should be considered as being part of the material in the skin sample/application site; if glue is used, the complete *stratum corneum* is removed by 1–2 strips; hence, the complete amount in the *stratum corneum* should be considered as potentially absorbed.)
- 7) As a minimum requirement, results from at least four replicates should be analysed in *in vitro* studies in line with the recommendations given in EC test guideline B.44 (*in vivo* dermal absorption). For statistical reasons, a larger number of replicates is preferred. SCCS (2010) recommends eight evaluable samples originating from four donors. The PPR Panel recommends for new studies to follow this approach for PPPs as well, particularly since animal welfare is not an issue for human skin samples.
 - (If less than four acceptable replicates are available, additional experimental data should be generated or one should revert to default values.)
- 8) Solubility of the test compound in the receptor fluid must be demonstrated as not being a rate limiting factor and should be at least 10 times higher than the expected (maximum) concentration of the test compound in the receptor fluid at the end of an *in vitro* study (see EFSA PPR Panel, 2011, section 3.3). Note that although confirming solubility in the receptor fluid is a requirement in EC test method B.45, it is often not reported.
 - (If test compound solubility in the receptor fluid is less than 10 times higher than its expected (maximum) concentration in the receptor fluid at the end of an *in vitro* study, the validity of the study is questionable.)
- 9) Exposure should mimic a working day (e.g. 6–10 h) with sampling for at least 24 h *in vitro* and a minimum of 96 h *in vivo* (see EFSA PPR Panel, 2011, section 3.5).
 - (If the exposure period is shorter, this can be compared with the lag-phase. If the product is removed before the end of the lag-phase is reached, the relevance of the study is in doubt. If the product is removed after the lag-phase is completed, it is possible to make a pro rata correction for the shorter duration, based on results of the linear phase. If sampling does not continue for an adequate period, all material in the skin sample/at the application site should be included in the determination of the dermal absorption value, or, if possible, absorption should be determined by extrapolating to an adequate time point.)
- 10) After the end of experimental exposure, the skin is washed and the characteristics of the skin rinsing step should be indicated in the report. The cleansing agent should be representative of normal hygiene practices (e.g. an aqueous soap solution).
 - (If washing is not performed, an overestimate of the absorption is likely to occur.)
- 11) When performing studies designed to permit correction of rat *in vivo* data with rat and human *in vitro* data, the *in vitro* and *in vivo* data should be as closely matched as possible in terms of rat strain used, exposure time, tested material, skin sample preparation, state of occlusion, vehicle, solvent/formulation volume per unit area and dilution rates/amount of a.i. per unit area (see Section 5.9 for details).
 - (If the rat and human *in vitro* studies and rat *in vivo* studies are not comparable, the human *in vitro* value can still be used on its own.)
- 12) Data should be presented for individual animals/replicates and as group means \pm standard deviations.
- 13) Maximum flux should be based on the calculation of the slope of the linear portion of the absorption: time curve and should not include the lag-phase or plateau.
- 14) If overall recovery is consistently low (mean over all animals or replicates < 95% for radiolabelled studies), this does not necessarily mean that the study cannot be accepted but an explanation must be provided as to why the missing material should not be considered

as absorbed. However, if the calculated dermal absorption value is < 5% and recovery < 95%, the missing amount should be added to the observed amount.

- [For rules on how to proceed when recovery (mass balance) is low (< 95%), see Section 5.2.]
- 15) To address variability between replicates, a multiple of the standard deviation should be added to the mean dermal absorption value (see Section 5.3).
- (If there is significant variation between replicates, see Section 5.3 for details.)
- 16) If non-radiolabelled material is used, the analytical methods used to determine the amount of absorbed material must be able to account for metabolism and hydrolysis, or data must be presented to permit back calculation from the analysed components to the amount of active substance absorbed (see Section 6.3 for details).
- (For non-radiolabelled studies, if the relationship between the recovered material and the amount absorbed cannot be demonstrated, a conservative default could be to assume that any deficit in the mass balance is absorbed material.)

5. Interpretation of studies

5.1. Tape stripping

See EFSA PPR Panel (2011), sections 3.4, 3.5 and 3.6.

Tape stripping is a procedure performed at the end of a dermal absorption study that involves the sequential application of adhesive tape to the area of skin that was exposed to a chemical (Trebilcock et al., 1994). If the tape strips are analysed individually, a profile of the chemical across the *stratum corneum* can be determined.

There has been a general practice within EFSA PRAPeR⁹ meetings to assume that the first 2 tape strips will represent material that will not become bioavailable due to desquamation. The PPR Panel proposes to follow this approach, that applies to both *in vitro* and *in vivo* studies (see EFSA PPR Panel, 2011, section 3.6).

Only if permeation (*in vitro*) or absorption (*in vitro*) is essentially complete at the end of the study, usually after a 24-h sampling period, can all tape stripped material be excluded from calculation of the absorbable dose fraction [see Section 5.6 option (i)]. This applies to both *in vitro* and *in vivo* studies (see Sections 5.5 and 5.7, and see EFSA PPR Panel, 2011, sections 3.4 and 3.5 providing the background for this statement).

For *in vitro* studies, permeation is considered essentially complete when > 75% of the amount that has permeated into the receptor fluid at the end of sampling (usually at 24 h) has reached the receptor fluid at the half time of the sampling period (usually at 12 h). For an *in vitro* study with a sampling period of 24 h, the mean relative permeation into the receptor fluid occurring within half of the sampling period (t_{0.5}) should be calculated from the individual replicate data on amounts recovered in receptor fluid (RF) at 12 h (RF₁₂) and 24 h (RF₂₄) as follows:

$$t_{0.5} = 100\% \times \sum_{i=1}^n \frac{RF_{12i}}{RF_{24i}} \times \frac{1}{n} \quad n = \text{number of valid replicates}$$

If the t_{0.5} value is close to 75%, a confidence interval should be estimated to demonstrate credibility of the conclusion that permeation is essentially complete. This can be achieved in analogy to the approach described in Section 5.4: a standard deviation is calculated from individual RF_{12i}/RF_{24i} ratios and multiplied by the k-value taken from Table 1. The lower limit of the 95% confidence interval is approximated as t_{0.5} (mean value) – ks, where s is the sample standard deviation. For *in vivo* studies where there is evidence that absorption is nearing completion, where less than 75% is absorbed (as described in Section 5.8), material from all tape strips can be excluded from the absorbed material if the evidence indicates that it is not bioavailable. However, this should be critically assessed on a case-by-case basis.

⁹ Peer Review of Pesticide Risk Assessment (carried out under the responsibility of EFSA's Pesticide Unit in the EU).

For both *in vitro* and *in vivo* studies, recovery for replicates and animals should be within the set limit (see section 5.2).

A template (Excel file) to support calculations (including t0.5) for dermal absorption from *in vitro* studies has been provided by BfR and published as supporting information. The Working Group (WG) confirms the template is in line with the requirements in the EFSA guidance and recommends using the template as part of the dossier.

5.2. Recovery

When recovery is below the set limit (mean over all animals/replicates < 95%), there is a need to consider whether the missing material should be considered as absorbed. It should be noted that the average should be calculated based on the recoveries determined for each individual animal/replicate. This also allows reporting a standard deviation for recovery, providing an indication for variability across replicates. The recovery value of 95% is stricter than that in the OECD test guidelines, but with modern analytical and pipetting techniques it is regularly achieved. A high level of recovery is required to support a low dermal absorption value; for example, if 9% of the test material is unaccounted for there is a high degree of uncertainty surrounding a proposed dermal absorption value of 1%. In such cases, additional evidence needs to be supplied to identify which element of the assay is associated with the missing material and that it is not specifically associated with material used in the calculation of absorption.

- 1) If overall recovery is consistently low (mean over all animals/replicates < 95% for radiolabeled studies), as a worst-case assumption, the missing material should be considered as absorbed and added to the absorbed amount, unless it can be justified that the missing material is unlikely to have been absorbed. Adding the missing material should certainly apply when the calculated dermal absorption value is < 5% and recovery is < 95%.
- 2) Alternatively, a 'normalisation' approach could be applied in which dermal absorption is expressed as a percentage of the total amount recovered (except when the calculated non-normalised dermal absorption is < 5%, then the addition rule stands). Values for all animals/replicates should be normalised individually. However, in principle, normalisation is the preferred option, critical evaluation of the available data needs to be performed to determine if significant amounts of the missing material could have been absorbed (e.g. from *in vivo* studies exhaled as CO₂). In such a situation, no normalisation is recommended, and consequently, the missing amount should be added to the absorbed amount.
- 3) If there are some replicates/animals with adequate recoveries, then the results for low and high recovery animals/replicates can be compared to those with adequate recovery to see if the losses are from absorbed or non-absorbed material.

Losses that are considered to be from non-absorbed material will have no impact on the results.

If losses appear to be from absorbed material, the values should be corrected for the losses by considering the lost fraction as absorbed.

If no clear conclusion can be drawn, only values from high recovery samples should be used to derive the absorption and replicates with low recoveries should be excluded entirely. However, as exclusion reduces the overall number of replicates, a balance must be found between uncertainty resulting from low recoveries and uncertainty from a lower number of acceptable replicates.

Low recovery (mean over all animals/replicates < 95%) is less of an issue when there is a high level of absorption as the impact will be proportionally lower. For volatile or potentially volatile compounds, measures should be taken to prevent loss (e.g. charcoal filter occlusion to achieve acceptable recovery despite volatilisation, see also Section 5.8).

5.3. Variability within the results and outliers

The preferred approach to addressing variability between replicates/animals is to add a multiple of the standard deviation to the mean value. The multiplication factor required depends on the number of replicates and is given in Table 1. The standard deviation of the absorption value should be calculated as indicated in Section 5.6 and the mean absorption as defined in Sections 5.6 and 5.8. The result of this calculation approximates the upper limit of the 95% confidence interval for the mean absorption (see Appendix B for the statistical justification). The use of the upper confidence limit

addresses uncertainty about mean absorption due to sampling variability. This approach is reasonably conservative and could reduce the need to repeat studies (particularly *in vivo* studies).

Table 1: Approach for the treatment of variability within the results

| Number of replicates (n) | Multiplication factor (k) |
|--------------------------|---------------------------|
| 4 | 1.6 |
| 5 | 1.2 |
| 6 | 1.0 |
| 7 | 0.92 |
| 8 | 0.84 |
| 9 | 0.77 |
| 10 | 0.72 |
| 11 | 0.67 |
| 12 | 0.64 |
| 13 | 0.60 |
| 14 | 0.58 |
| 15 | 0.55 |
| 16 | 0.53 |

Values of k were calculated assuming an underlying normal distribution as an approximation for variability and random sampling.

Thus, to address variability between replicates/animals, dermal absorption should be calculated as follows: Absorption (mean value) + ks, where s is the sample standard deviation.

Clear statistical criteria to define outliers to be considered for removal should be provided in the study report, taking into account the tendency of absorption data to be skewed. However, outliers should not be removed on statistical grounds alone; a plausible cause for the value being an outlier should be put forward, e.g. a membrane damaged during the experiment. If not, it should be considered part of normal experimental variation and not be left out. Once a replicate has been excluded based on an outlying parameter value, it should be excluded from calculations for all other parameters as well. Reasons for excluding outliers should be clearly stated in the study report and summary text. In addition, the full results from the samples considered to be outliers must be presented. It should be noted that results treated as outliers should include spuriously low values as well as high ones.

5.4. Rounding of values

Dermal absorption studies tend to have a relatively high level of variability associated with the results. So as not to imply spurious precision, final dermal absorption (i.e. after pro-rata or triple pack corrections) values should be rounded to a maximum two significant figures.

For example:

0.098% = 0.098%

0.268% = 0.27%

1.839% = 1.8%

14.86% = 15%

56.77% = 57%

85.22% = 85%

5.5. Dilution rates (tested concentrations)

See flow chart 3.

The concentration(s) tested should cover the extremes of those recommended on the product label. If the lowest concentration tested is greater than the lowest concentration of the same formulation recommended on the label, consideration should be given to increasing the dermal absorption pro rata to account for any limitation of absorption due to the amount of material applied to the test site. However, if the dermal absorption from the concentrate, a dilution and the lowest tested concentration (second dilution) shows no indication of concentration related absorption, then there is no need to increase the value for the lower (untested) concentration of the same formulation recommended on the label.

Pro-rata correction assuming a proportional response is considered to be a conservative but appropriate approach in the absence of data and is a concept of worst-case linear extrapolation which applies to dilutions (see also Appendix B).

In case the same formulation and its dilution(s) are tested, this approach can be used to derive dermal absorption values for other dilutions with concentrations of the same formulation lower than the highest dilution (lowest tested active substance concentration). The linearity is assumed from the origin (0,0) to the highest dilution (lowest tested active substance concentration). Pro-rata correction should not be used to derive dermal absorption values for dilutions with higher concentrations (e.g. extrapolation back from a dilution towards the concentrate or from a dilution towards a less diluted formulation). In that case, the dermal absorption from the tested dilution should apply (the closest experimental tested dilution). It should be noted that if the pro rata correction gives a value above the default value for dilutions (see Section 6), then the respective default value should apply.

The following example is presented to clarify some of the conditions to be fulfilled:

Default approach – difference between dilutions

Dermal absorption of 1 + 50 dilution = 12%.

Highest label dilution is 1 + 80 for which a value of $12 \times 80/50 = 19\%$ can be derived using the pro-rata correction.

Correction necessary.

Refinement of the default approach is possible if it is demonstrated, e.g. that there is no change of dermal absorption within the relevant dilution/concentration ranges. The corresponding justification should address at least the following points:

- data justifying such a conclusion;
- statistical uncertainty in measured values on which the conclusion is based.

5.6. *In vitro* studies¹⁰

Flow chart 4a, see EFSA PPR Panel (2011), section 3.1.

Human skin samples provide the best estimate of dermal absorption. If non-human skin is used, then the rat would be recommended for consistency reasons. If a dermal absorption study with rat or human skin samples has been well performed (see Section 4 and EC test method B.45), the dermal absorption should be calculated on the following basis using mean values:

i) when:

- the sampling period is 24 h

and

- it can be concluded with confidence that over 75% of the permeation (to be calculated as described in Section 5.1) occurred within half of the duration of the total sampling period

Absorption = receptor fluid + receptor chamber washes + skin sample (excluding all tape strips)

ii) when:

- the sampling period is less than 24 h

or

- less than 75% of the absorption occurs within half the duration of the study

then

Absorption = receptor fluid + receptor chamber washes + skin sample (excluding tape strips 1 and 2)

If tape stripping has been performed with strips being pooled, then all tape strips should be included in the absorbed material.

When calculating means and standard deviations for the absorption values, absorption should be calculated for each individual replicate, and the average and standard deviations should be determined using these absorption values for individual replicates.

¹⁰ Use of reconstituted skin is not recommended, see Section 4.

To address variability between replicates, a multiple of the standard deviation should be added to the mean dermal absorption value (see Section 5.3).

A template (Excel file) to support calculations for dermal absorption from *in vitro* studies has been provided by BfR and published as supporting information. The WG confirms the template is in line with the requirements in the EFSA guidance and recommends using the template as part of the dossier.

5.7. Non-human primates *in vivo*

Flow chart 4b, see EFSA PPR Panel (2011), section 3.9.

The use of non-human primates is not recommended; however, the following guidance is provided for the evaluation of existing studies.

In order to use *in vivo* data on non-human primates the following points need to be addressed:

- A minimum group size of 4 should be used (this is in line with EC test method B.44). If smaller numbers are used, then the highest result, rather than the mean, should be chosen.
- The application site should be one that gives a realistic level of dermal penetration (e.g. forearm, torso, forehead). Correction for mass balance needs to be done if non-human primates were not sacrificed at the end of the study and non-radiolabelled material was used.
- The analyses need to cover possible metabolites or use a marker compound(s) that can be back extrapolated to absorbed material based on the amount in urine, faeces and exhaled air.
- It is not usually possible to determine the residue in the dermis at the application site or distributed within the body.
- Compare the excreted material following oral or intravenous and dermal dosing. The dermal study should include a long enough sampling duration to confirm that excretion is essentially complete. For example, if 25% of an intravenous dose is detected in excreta using a particular analytical technique and 5% of a dermal dose is detected in excreta then the dermal absorption can be considered to be 20% ($5\% \times 100/25$).
- Exclude an extensive first pass metabolism, incomplete absorption or extensive biliary excretion. A first tier approach would be to assume 100% oral absorption, and determine the ratio of the amount detected in urine in the dermal study with the amount in urine from the oral study.
- Take blood samples and determine the ratio of the area under the curve (AUC) from intravenous or oral dosing with that from an equivalent dermal dose. The default assumption is 100% absorption from the oral route: e.g. $(\text{AUC dermal}/\text{AUC oral}) \times 100 = \% \text{ dermal absorption}$.

It is the responsibility of the notifier to present a justification for the analytical method used and how the recovered material relates to the amount actually absorbed. Alternatively, as a conservative approach, all material not recovered in the skin washes plus the first two tape strips (if performed) can be considered as absorbed.

5.8. Rat¹¹ *in vivo*

Flow chart 4b, see EFSA PPR Panel (2011), section 3.4.

If a dermal absorption study in rats has been well performed [see OECD test guideline 427 (OECD, 2004a)], the dermal absorption should be calculated on the following basis using data from the terminal sampling time:

i) When:

- the sampling period is 24 h or longer

and

- it can be concluded with confidence that over 75% of the total absorption (material in excreta, exhaled gasses and in the carcass at the end of the study) occurred within half of the duration of the total sampling period (may be evaluated in analogy to the approach described in Section 5.1 for *in vitro* studies)

then

Absorption = excreta + CO₂/volatiles (in exhaled air) + carcass + skin (excluding tape strips)

¹¹ Rat or any other non-primate experimental animal.

ii) When:

- the sampling period is less than 24 h

or

- less than 75% of the absorption (as described above) occurs within half the duration of the study

then

Absorption = excreta + CO₂/volatiles (in exhaled air) + carcass + skin (excluding tape strips 1 and 2)

To address variability between replicates/animals, a multiple of the standard deviation should be added to the mean dermal absorption value (see Section 5.3).

If tape stripping has been performed with strips being pooled, then all tape strips should be included in the absorbed material.

- iii) In cases where the evidence indicates that absorption was essentially complete at the end of the study (e.g. marked decline in the amount over the last three sampling times), but the criteria in option (i) above were not met, a case-by-case consideration of the potential bioavailability of the application site residue can be made. This should take account of factors such as whether the remaining material is in the outer layers of the *stratum corneum* and the duration of the study. For example, if the study was run for only 96 h and the majority of the residual material is in tape strips from the lower layers of the *stratum corneum*, then the material is probably bioavailable. If the study was run for 168 h and the majority of the material is in the tape strips from the upper layers of the *stratum corneum*, it is reasonable to exclude these tape strips.
- iv) Poor recovery in *in vivo* studies can be due to a variety of reasons that can be investigated further using data normally available.
- If the exhaled volatiles have not been measured and the radiolabel is on a part of the molecule that could be cleaved and give rise to CO₂ and/or exhaled volatiles, it is reasonable to assume that some or all of the missing material was absorbed and lost via exhalation. Some information on exhaled volatiles/CO₂ is often available from the oral absorption, distribution, metabolism and excretion (ADME) data (assuming the same radiolabel position is used). If no volatiles or CO₂ were detected in the oral ADME study, it is reasonable to conclude that this route will not be the reason for poor recovery following dermal exposure.
 - Desquamation might be a cause of poor recovery particularly if the duration of the study is 7 days or longer. If a significant amount of material is removed by the final swabbing and/or is present in the first tape strip, this could support an argument that the poor recovery is due to desquamation and the missing material was not absorbed. On the contrary, if there is only a small amount of material obtained with the final swabbing or the first tape strip, it is unlikely that desquamation would have been a cause of significant loss.

In all cases of poor recovery (mean < 95%), reasoning why the missing material should not be considered as absorbed, has to be presented (see Section 5.2).

5.9. Integration of *in vivo* and *in vitro* data

See EFSA PPR Panel (2011), section 5.4.

To ensure scientific validity, it is essential that the study protocols for the human and rat skin *in vitro* studies are well matched for variables that could influence the results, for example:

- Skin type (i.e. split-thickness)
- Test material/formulation/vehicle/concentration/amount per unit skin area of the active substance
- State of occlusion, exposure duration and sampling period
- Receptor fluid composition
- Swabbing technique
- Analytical techniques

Also, the results should meet the quality criteria set above, in particular with respect to variability, number of acceptable replicates and recovery (see Sections 5.2 and 5.3).

In the *in vivo* study, the same test material/formulation/vehicle/area dose (concentration) and a similar exposure time and swabbing technique should be used as in the *in vitro* studies.

Normally, this will be achieved by performing the studies contemporaneously at the same test facility; however, this is not an essential requirement. If the human and rat skin *in vitro* studies are not well matched, then the possibility of a comparison of the relative dermal absorptions should be very carefully evaluated.

If the *in vitro* studies are closely matched to each other and to the *in vivo* study, the *in vivo* human dermal absorption can be derived based on the following equation:

$$\text{In vivo human absorption} = \frac{[(\text{in vivo rat absorption}) \times (\text{in vitro human absorption})]}{(\text{in vitro rat absorption})}$$

The calculation can be based on either % absorption (option 1) or flux (option 2).

Option 1:

- Calculate % absorption.
- The relative absorption can be estimated by taking the ratios of the % absorption.

Then, the derived human *in vivo* value will be:

$$\text{in vivo human \% absorption} = \frac{\text{in vivo rat \% absorption}}{\text{in vitro rat \% absorption}} \times \text{in vitro human \% absorption}$$

Option 2:

- Calculate the maximum flux, normally from a linear portion, of 2 h or longer, of the absorption-time curve. A shorter time period may be used if absorption is very rapid and essentially complete within 4 h.
- The relative absorption can be estimated by taking the ratios of the maximum flux.

Then, the derived human *in vivo* value will be:

$$\text{in vivo human \% absorption} = \frac{\text{in vitro human flux rate}}{\text{in vitro rat flux rate}} \times \text{in vivo rat \% absorption}$$

There are circumstances when the flux might not be appropriate (e.g. the linear phase is significantly longer in the human samples). In these cases, the comparison can be performed using the % absorbed, but it must have the same basis for both rat and human samples (i.e. in terms of inclusion of tape strip material or residue in the skin sample).

A template (Excel file) to support calculations for dermal absorption from *in vitro* studies has been provided by BfR and published as supporting information. The WG confirms the template is in line with the requirements in the EFSA guidance and recommends using the template as part of the dossier.

5.10. Choice of dermal absorption values for worker/resident exposure

Until the outcome of the ongoing research into this aspect is available and conclusions have been drawn, it is proposed that the appropriate dermal absorption value for exposures to dried dispersed residue should be the higher of the values for the concentrate and the in-use dilution.

If an acceptable estimate of worker/resident exposure cannot be obtained using this approach, specific evaluations could be performed on a case-by-case basis. These could take into account factors such as the level of the dislodgeable foliar residue (mass/unit area) and transfer coefficient vs the loading used in the dermal absorption studies with concentrate and dilution(s) to help determine the most appropriate dermal absorption value to use. Any lowering of default values commonly applied in exposure models should be justified.

5.11. Use of data from field studies

Dermal absorption estimated in field monitoring studies of workers/operators is rarely accurate because of difficulties in measuring skin deposition and of knowing metabolism in humans. Therefore, data obtained in these studies can only be used to support experimentally determined values.

6. How to proceed when there are no data on the formulation under consideration

This approach is outlined in flow chart 1a. When using it, care should be taken to ensure that the outcome is relevant for the product under consideration.

6.1. Default values

Based on the evaluation of new human *in vitro* data, indicating a significant impact on dermal absorption of concentration status ('concentrate' or 'dilution') and formulation category, the following default values are recommended (see Table 2; Appendices A and B for details).

A default dermal absorption value of 25% may be applied for concentrated products that are organic solvent-formulated^(a) or in other^(b) types of formulations.

A default dermal absorption value of 10% may be applied for concentrated products that are water-based/dispersed^(c) or solid-formulated^(d).

A default dermal absorption value of 70% may be applied for (in use) dilutions of organic solvent-formulated^(a) or in other^(b) types of formulations.

A default dermal absorption value of 50% may be applied for (in use) dilutions water-based/dispersed^(c) or solid-formulated^(d).

Table 2: Default values to be used in the absence of experimental data

| Formulation category | Concentration status | Default value |
|--|----------------------|---------------|
| Organic solvent-based ^(a) or other ^(b) | Concentrate | 25% |
| | Dilution | 70% |
| Water-based/dispersed ^(c) or solid ^(d) | Concentrate | 10% |
| | Dilution | 50% |

(a): Formulation types: emulsifiable concentrate (EC), emulsion, oil in water (EW), suspo-emulsion (SE), dispersible concentrate (DC), oil miscible liquids (OL/OF), oil-based suspension concentrates (OD), emulsion for seed treatment (ES), microemulsion (ME).

(b): Formulation types: bait concentrate (CB), capsule suspension (CS), gel for direct application (GEL/GD), bait, ready for use (RB), mixture of capsule suspension and suspension concentrate (ZC), seed coated with a pesticide (PS), experimental solution of active substances in solvent (AI).

(c): Formulation types: soluble concentrate (SL), suspension concentrate (SC), flowable concentrate for seed treatment (FS), flowable (FL) (=SC).

(d): Formulation types: wettable powder (WP), water-dispersible granules (WG/WDG), water-soluble granules (SG), water-soluble powder (SP), powder for dry seed treatment (DS).

The grouping of formulation types into categories used for the statistical estimates intended to support the setting of default values (see Table B.2 presented in Appendix B) is based on information on the chemical composition of the tested product, information on the phase in which the active substance is dissolved or emulsified/suspended and the expectable impact on dermal absorption. To select the appropriate default, the 'Manual on development and use of FAO and WHO specifications for pesticides' should be used for categorisation of formulations to select the appropriate default (FAO/WHO, 2016).

The categorised groups were organic solvent-based, water-based/dispersed, solids and other formulation types. Default values are set for concentrated products¹² and for their (in use) dilutions (see above). If special formulation types are not contained in the data set and cannot be grouped with the proposed categories the worst-case default values should be adopted (solvent based + other). Ready-to-use products have to be categorised as well. A ready-to-use product might be a dilution of a concentrated product sold in a smaller packaging for non-professional users. In this case, it is reasonable to categorise this product as a dilution of the respective concentrated formulation. Gel baits or slug pellets, for example, should be categorised as 'other' because these are ready for use (RB) formulations and the compositions are clearly different from solid formulations.

6.1.1. Consideration of the oral absorption value when setting a default value

Flow chart 1b, see EFSA PPR Panel (2011), section 5.1.

¹² Products as placed on the market.

In exceptional cases, if oral absorption is less than 70% for organic solvent-based or other formulations or less than 50% for water-based/dispersed or solid formulations, this can be used as a surrogate dermal absorption value for (in-use) dilutions. If oral absorption is less than 25% for organic solvent-based or other formulations or less than 10% for water-based/dispersed or solid formulations, it can be used instead of the default value for concentrated products. There are usually no oral ADME studies for formulations that include co-formulants which are possibly modifying dermal absorption. For these reasons, estimates based on oral absorption should be applicable in only a limited range of circumstances after careful consideration of doses and vehicle used in the ADME studies, where bile-cannulation was also performed.

6.2. Use of data on similar formulations

Flow chart 5, see EFSA PPR Panel (2011), sections 2.6 and 4.4.

Dermal absorption data on another (reference) formulation can be used if the formulation for which dermal absorption needs to be determined is closely related. This occurs when all the following conditions are met:

- Content of relevant components in the formulation to be assessed (e.g. other active substance, synergist, safener, wetting agent, surfactant, solvent, emulsifier, preservative, stabiliser, detergent, adhesive, antifreezing substance (= all co-formulants), similar chemical types of co-formulants might be grouped as described below) is within permitted variation ranges of those in the reference formulation. The permitted ranges are provided in Table 3. The given concentration ranges and permitted variations are based on the bridging principles for hazard assessment of mixtures provided by the Regulation (EC) No 1272/2008¹³ and the guidance on significant and non-significant changes (Sanco/12638/2011, 20 November 2012 rev.2), respectively.

Table 3: Permitted variation for similar formulation

| Initial concentration range of the constituent (% w/w) | Permitted (relative) variation (%) |
|--|------------------------------------|
| ≤ 0.5 | ±100 |
| ≤ 1.0 | ±50 |
| ≤ 2.5 | ±30 |
| 2.5 < c ≤ 10 | ±20 |
| 10 < c ≤ 25 | ±10 |
| 25 < c ≤ 100 | ±5 |

c: concentration.

Addition of substances not contained in the reference formulation might be acceptable up to a concentration of ≤ 0.5%, but only if it is shown or scientifically justified that this minor change does not have an impact on physical–chemical or toxicological properties of the formulation. In individual cases, greater variations might be acceptable, for example, replacement of a co-formulant by water or increase of an inert compound.

- Co-formulants of both formulations are chemically and physicochemically closely related (e.g. toluene vs xylene; octanol vs nonanol; e.g. linear alkyl sulfonate is not replaced by an aromatic sulfonate derivative) as well as with respect to interaction with the active substance (e.g. solubility or enhancement of toxicological properties). However, the evaluation of similarity should be expert judgement on a case by case basis.
- Additional active substances do not possess properties that may change skin permeability (e.g. irritant and sensitising properties).
- Formulation is of the same or lower skin irritancy based on scores in studies. These must include initial findings (as dermal absorption is often significant within the first 24 h), not just the classification. If no skin irritation study is available, a comparison based on the irritancy of the components can be performed, but the outcome should be interpreted with care as classification does not take initial irritation scores into account.

¹³ Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. OJ L 353, 31.12.2008, p. 1–1355.

- Formulation having the same or no sensitising potential based on classification.
- Active substance concentration is within permitted variations of that in the reference formulation as given in Table 4, based on the FAO and WHO specifications for pesticides (FAO/WHO, 2016, chapter 4.3.2)¹⁴

Table 4: Permitted variation for active substance in similar formulations (from 'Manual on development and use of FAO and WHO specifications for pesticides', 2016)

| Initial concentration range of the constituent (% w/w) | Permitted (relative) variation (%) |
|--|--|
| ≤ 2.5 | ±15 for homogeneous formulations (EC, SC, SL), or ±25 for heterogeneous formulations (GR, WG) |
| 2.5 < c ≤ 10 | ±10 |
| 10 < c ≤ 25 | ±6 |
| 25 < c ≤ 50 | ±5 |
| ≥ 50 | ±2.5 |

c: concentration; EC: emulsifiable concentrate; SC: suspension concentrate; SL: soluble concentrate; GR: granules; WG: water-dispersible granules.

- If it is demonstrated that the percentage dermal absorption is inversely related to the concentration of the active substance, a dermal absorption value of a lower concentrated formulation (dilution) could apply to formulations containing higher amounts of the active substance if the other conditions are fulfilled.

It is considered unlikely that the above criteria will be met when moving from one formulation type to another (e.g. suspension concentrate to emulsifiable concentrate).

- In analogy to OECD Guidance Notes on dermal absorption (2011), a multi-to-one approach might be acceptable in exceptional cases. It has to be demonstrated that dermal absorption of a variety of products is always in the same range and that the formulation of the product under evaluation is covered by the tested formulations in order to accept an overall value based on a multi-to-one approach.

6.3. Data on the active substance

Flow chart 6.

Data generated with the unformulated active substance should only be used when the formulation under evaluation is very closely related to the vehicle used in the study with the active substance, in terms of solvent, surfactant content, skin irritancy and active substance content.

6.4. Microencapsulated formulations

See EFSA PPR Panel (2011), section 5.6.

The dermal absorption values used in exposure assessments should, by default, be based on the product within the capsules. If acceptable exposure assessments cannot be achieved with this approach, a case-by-case evaluation based on the properties of the encapsulated product relevant to specific exposure scenarios can be performed:

- If the capsule is shown to remain intact within the formulation over 2 years (standard storage stability test), then any exposure to the concentrate during mixing and loading can be assumed to be to the encapsulated product and the dermal absorption value for the encapsulated concentrate can be used, if available.
- If the capsule is seen to remain intact on dilution and throughout the application process (e.g. through pressurised spray nozzles), then exposures relating to the time of application can be based on the dermal absorption value for the diluted encapsulated product, if available.
- Dermal absorption values of the product within the capsule should be used if the integrity of the capsule has not been demonstrated and in all cases for re-entry workers and residents (see Section 5.10) since they are likely to be exposed to the material within the capsules that

¹⁴ http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/Specs/JMPS_Manual_2016/3rd_Amendment_JMPS_Manual.pdf

needs to be released in order to be effective. Should dermal absorption studies be available for the active substance both in a concentrated solution and in a dilution, the higher of the two figures should be used for assessment of dermal exposure for workers and residents.

6.5. Use of other information

6.5.1. QSARs and other mathematical models

See Appendix D.

The use of existing QSAR approaches for prediction of dermal absorption of active substances from pesticide products should be evaluated using a homogeneous data set of PPPs (e.g. according to OECD criteria). Until after such an evaluation a good predictive model has been identified, the use of QSARs or other mathematical models for the prediction of dermal absorption is not recommended.

6.5.2. Information on related active substances (read across)

See EFSA PPR Panel (2011), section 5.5.

The use of data on related active substances needs to be considered on a case-by-case basis, taking account of the properties of the active substances and formulations and the uncertainties in the data sets. If such an approach is applied, sufficient reasoning must be provided.

Molecules of very similar structure (e.g. ethyl substituent replacing methyl) and physicochemical properties would be expected to have similar dermal absorption characteristics in the same co-formulants.

Similarly, it might be possible to interpolate data from a number of similar active substances for drawing conclusions on a new active substance that is within the series. For instance, if the dermal absorption of a group of five closely related active substances in similarly formulated products is between 15% and 20%, it is reasonable to conclude that a sixth compound in the series would have a dermal absorption of around 15–20% if it has a similar formulation. However, it would not be appropriate to extrapolate data from a series of three closely related compounds with dermal absorption values of 2–12% based on significantly different formulations.

6.5.3. Comparison of oral and dermal toxicity data

It is not recommended to derive the dermal absorption of a compound by comparing the toxicity produced at different dose levels via the oral and dermal routes.

For the limitations, conditions and circumstances when this approach might be applied see the EFSA PPR Panel (2011), section 5.2.

7. Data presentation in assessment reports

In order to aid the independent evaluation of dermal absorption data, without needing to go back to the full study report, it is recommended that, as a minimum, the information given in Table 5 is presented.

Table 5: Template with minimum information on dermal absorption studies to be presented in assessment reports

| <i>In vitro and in vivo studies</i> |
|---|
| Material/product tested (name/code number) |
| Type of formulation |
| Concentration of active substance in the formulation |
| Vehicle used (if any) |
| Dilution rates |
| Surface area dose in micrograms of active substance per cm ² |
| Exposure time |
| Sampling duration (time of last sample) |
| Animal species/strain and skin sample source/application site |
| Group size/number of replicates/donor's ID for replicate |
| Total recovery (individual values for replicates, mean values ± SD) |

***In vitro* and *in vivo* studies**

Amount absorbed (individual values for replicates, mean values \pm SD)

Samples contributing to the amount absorbed and samples removed as outliers (with justification)

Type of tape strip used

***In vivo* studies**

Amount in excreta (individual values, mean values \pm SD)Amount in carcass (individual values, mean values \pm SD)Amount in exhaled volatiles/CO₂ (individual values, mean values \pm SD)

75% excreted in first half of study?

Amount in stripped application site (individual values, mean values \pm SD)Amount in tape strips 3 to ∞ (individual values, mean values \pm SD)Amount in tape strips 1 + 2 (individual values, mean values \pm SD)Amount in application site washes (individual values, mean values \pm SD)

Swabbing

***In vitro* studies**

Type of diffusion cell

Receptor fluid composition

Specification of solubility in receptor fluid as recommended

 $t_{0.5}$ valueAmount in receptor fluid and chamber wash (individual values for replicates, mean values \pm SD)Amount in stripped skin sample (individual values for replicates, mean values \pm SD)Amount in tape strips 3 to ∞ (individual values for replicates, mean values \pm SD)Amount in tape strips 1 + 2 (individual values for replicates, mean values \pm SD)Amount in skin sample washes (individual values for replicates, mean values \pm SD)

Swabbing

SD: standard deviation.

Modifications to this template will be required to match study designs, e.g. if tape stripping is not performed or if all the strips are pooled.

A template (Excel file) to support calculations for dermal absorption from *in vitro* studies has been provided by BfR and published as supporting information. The WG confirms the template is in line with the requirements in the EFSA guidance and recommends using the template as part of the dossier.

8. Flow charts

Flow chart 1a: Procedure to select default absorption values.

Flow chart 1b: Procedures to follow when there are no dermal absorption data on the actual formulation under evaluation.

Flow chart 2: General procedure for decision of dermal absorption value of plant protection products.

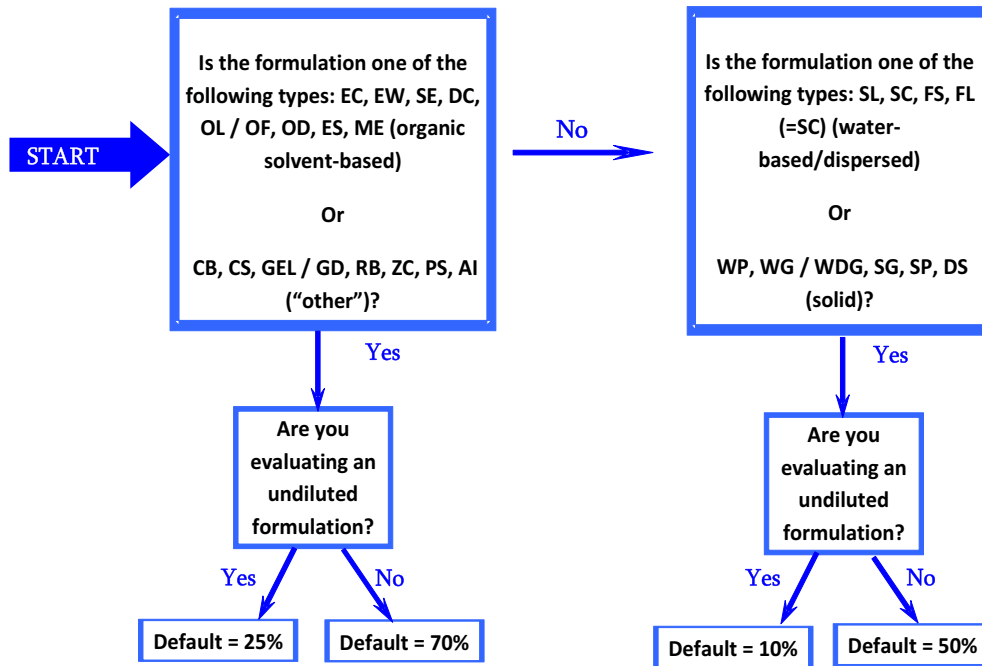
Flow chart 3: Procedures to follow when using dermal absorption data generated at dilutions different to those representing 'in use' conditions.

Flow chart 4a: Consideration of *stratum corneum* and application site residues *in vitro*.

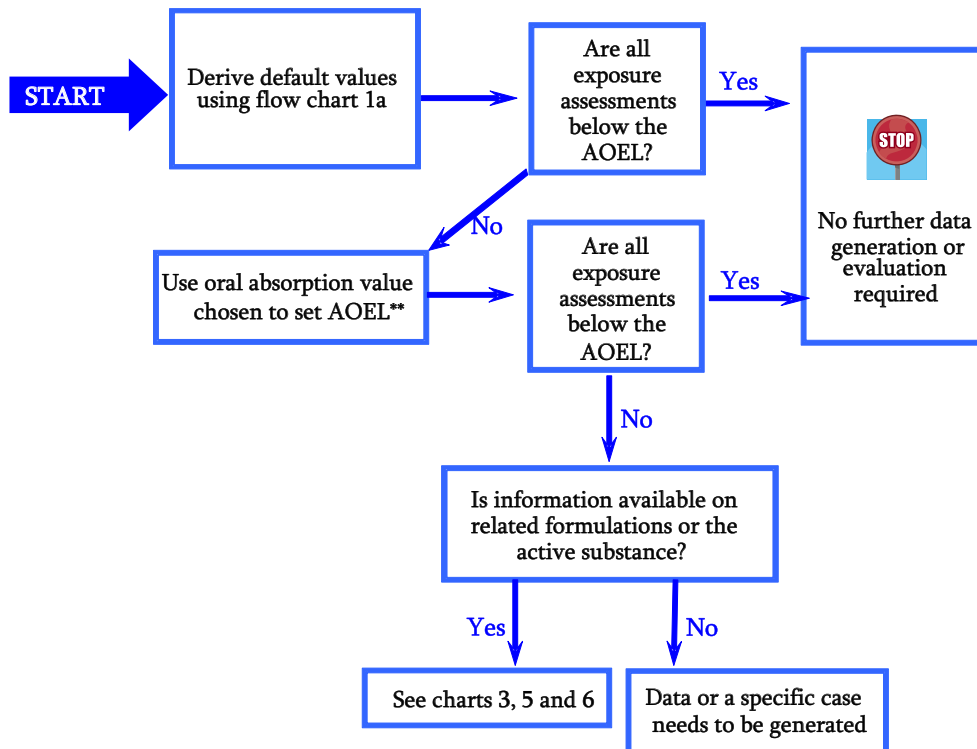
Flow chart 4b: Consideration of *stratum corneum* and application site residues *in vivo*.

Flow chart 5: Procedures to follow when reading across dermal absorption data between formulation types.

Flow chart 6: Procedures to follow when extrapolating dermal absorption data on an active substance to a formulated product.



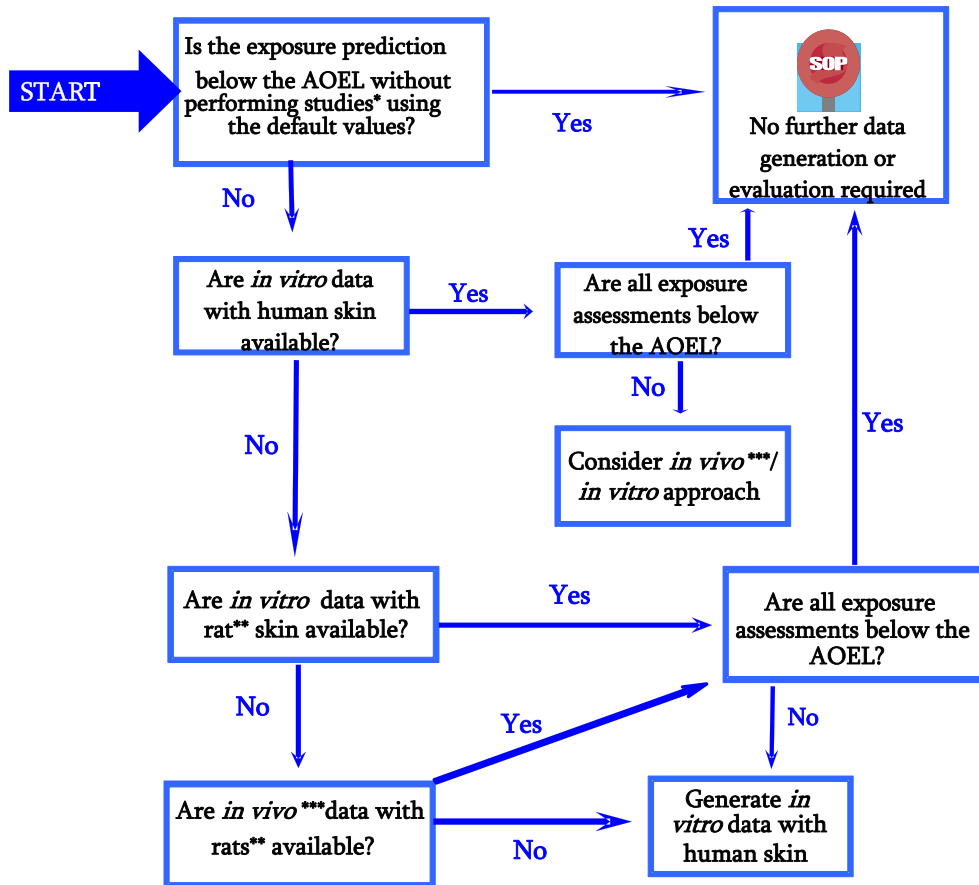
Flow chart 1a: Procedure to select default absorption values



* a.s.: active substance.

** Approach acceptable only in limited range of circumstances (see Section 6.1.1).

Flow chart 1b: Procedures to follow when there are no dermal absorption data on the actual formulation under evaluation

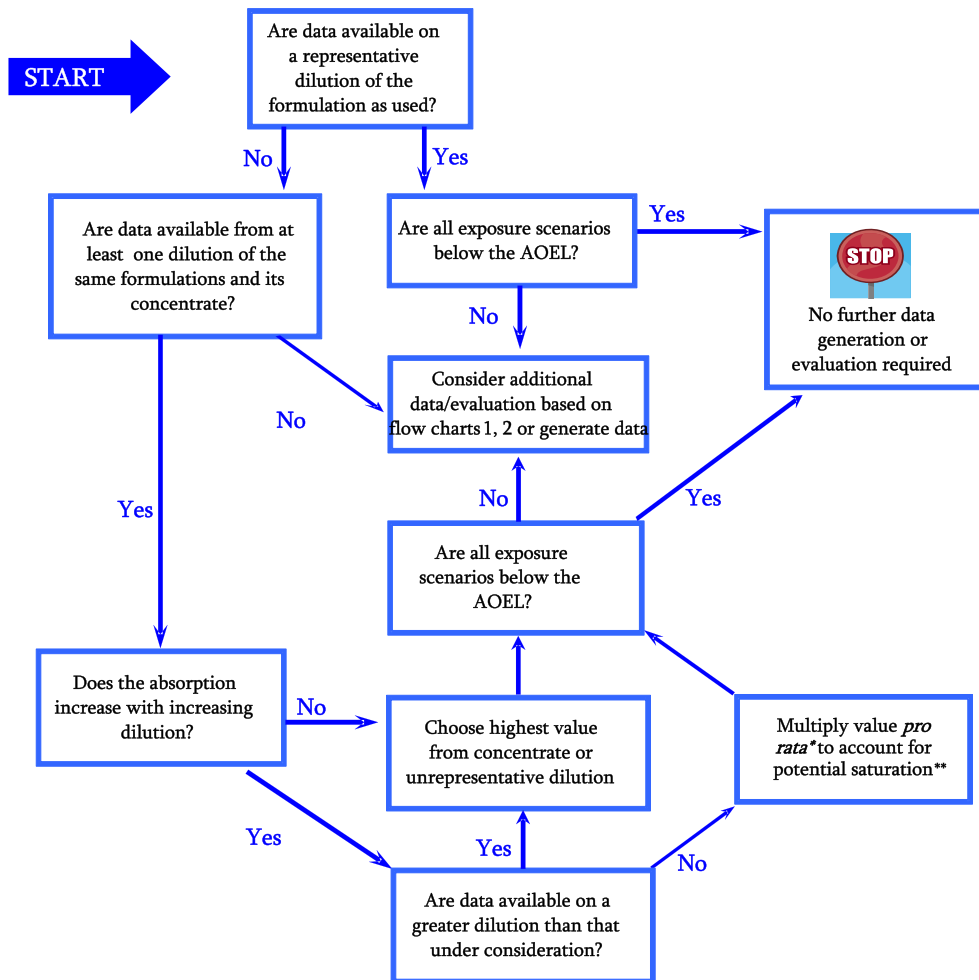


* Studies should be on a representative formulation.

** Or other species.

*** Existing *in vivo* studies.

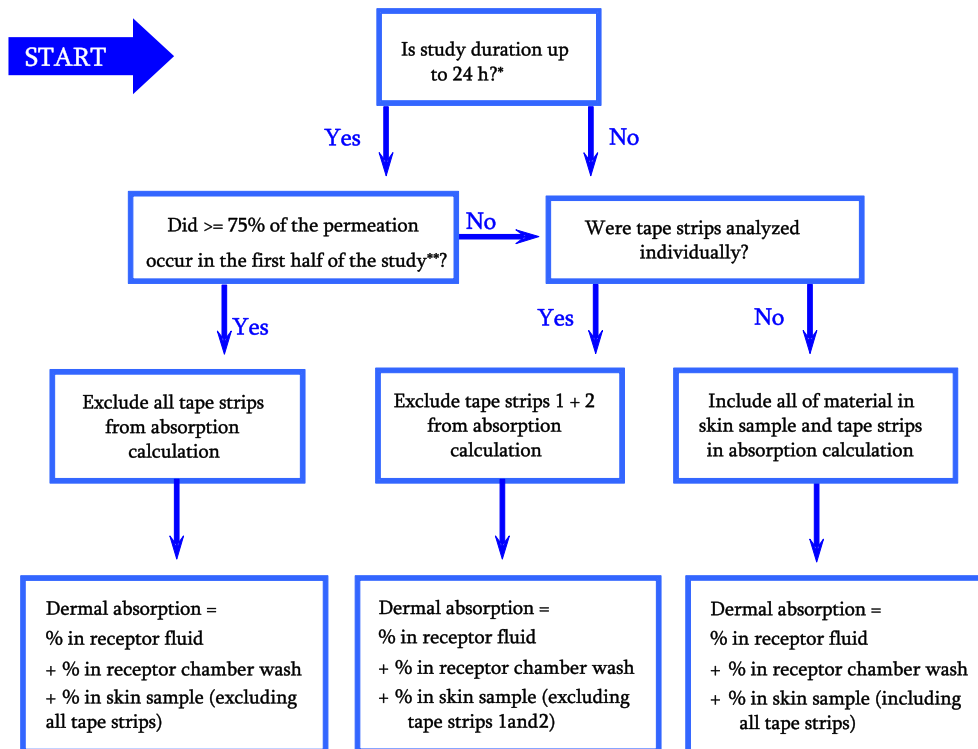
Flow chart 2: General procedure for decision of dermal absorption value of plant protection products



* e.g. if data are for 1 + 300 dilution but required dilution is 1 + 1200, multiply by 4.

** If the multiplication yields a higher result than the default value from dilutions of the respective formulation type considered, one may use that default value.

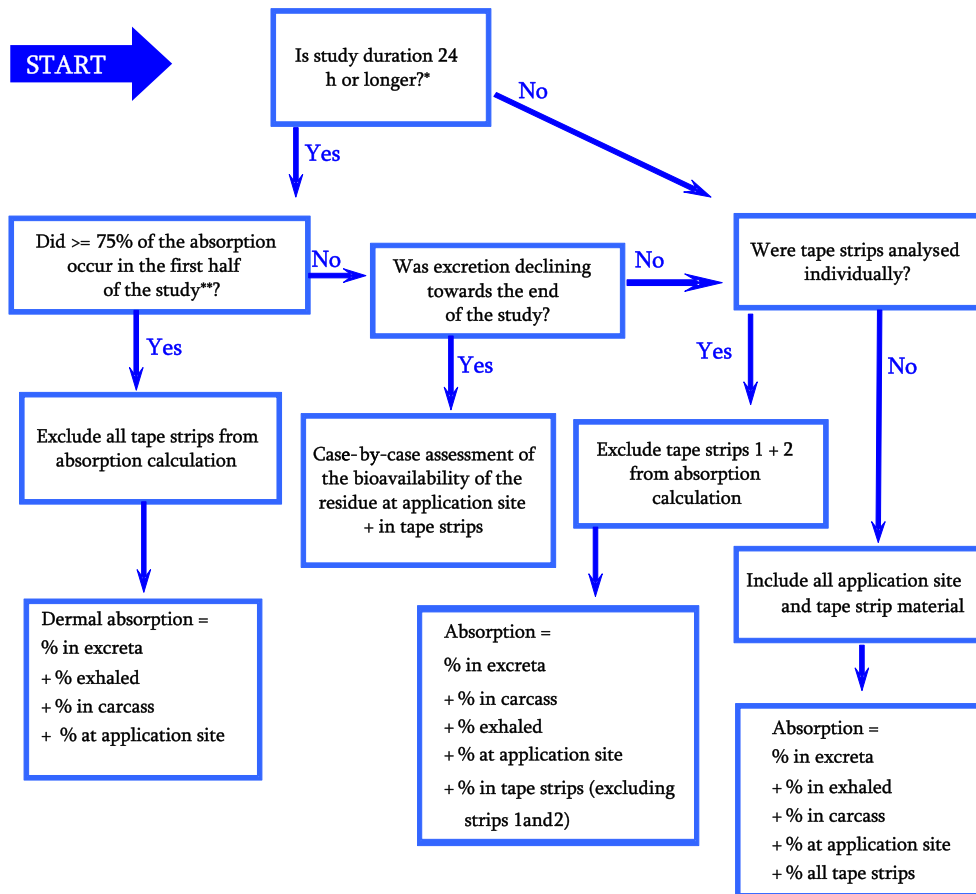
Flow chart 3: Procedures to follow when using dermal absorption data generated at dilutions different to those representing 'in use' conditions



* Duration refers to the study and not to exposure of skin.

** And variability within results is addressed as reported in Sections 5.1 and 5.3.

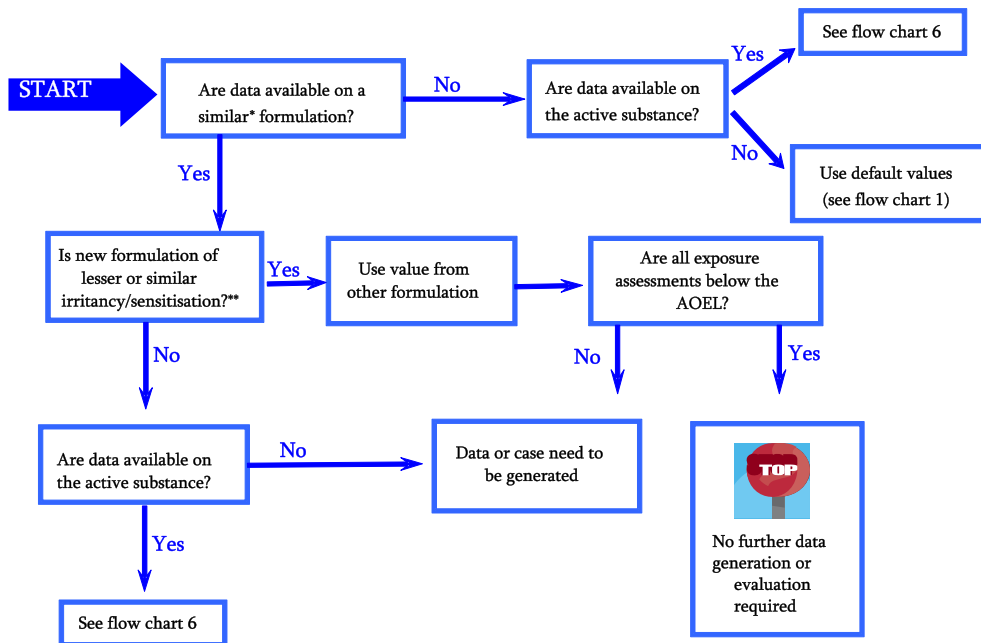
Flow chart 4a: Consideration of *stratum corneum* and application site residues *in vitro*



* Duration refers to the study and not to exposure of skin.

** And variability within results is addressed as reported in Sections 5.1 and 5.3.

Flow chart 4b: Consideration of *stratum corneum* and application site residues *in vivo*



* See Section 6.2 for details of when formulations can be considered similar.

** Based on scores, not just classification for irritation. Consider classification for sensitisation.

Flow chart 5: Procedures to follow when reading across dermal absorption data between formulation types



*or a simple aqueous vehicle, e.g. 1% carboxymethylcellulose.

**Based on scores not just classification for irritation. Consider also classification for sensitisation.

Flow chart 6: Procedures to follow when extrapolating dermal absorption data on an active substance to a formulated product

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Glossary and Abbreviations

| | |
|----------------------------|---|
| ADME study | Absorption, distribution, metabolism, excretion study. |
| Area under the curve (AUC) | Area under the plasma drug/chemical concentration versus time curve; a measure of drug/chemical exposure. |
| Dermal absorption | The movement of a chemical from the outer surface of the skin into the circulatory system, eventually leading to systemic exposure towards the chemical (dermal bioavailability) and its metabolites. Also called as percutaneous absorption. |
| Dermal penetration | The movement of a chemical from the outer surface of the skin into the epidermis, but not necessarily into the circulatory system. |
| Dermal permeation | The penetration through one layer into another, which is both functionally and structurally different from the first layer. |
| Flux | The amount of material crossing a defined area in a set time. A chemical with a high dermal flux will be absorbed more readily than a chemical with a lower flux. |
| Full-thickness skin | Full-thickness skin preparations consist of a 500–1000 μm thick skin sample, incorporating the <i>stratum corneum</i> , viable epidermis, and dermis. |
| Lag-phase | The time taken for the absorption of a chemical across the skin to reach a linear flux. Can be determined by extrapolating the line of linear flux back to the intercept at the X-axis of an absorption: time plot. |

| | |
|------------------------|---|
| Log P_{ow} | The logarithm of the partition coefficient of a substance between octanol and water (i.e. the relative maximum amount of a chemical that will dissolve in octanol and in water). A compound with a solubility of 100 g/L in octanol and 1 g/L in water would have a log P_{ow} of 2.0. |
| Split-thickness skin | Split-thickness (dermatomed) skin consists of 200–400 μm thick sample, in which the lower dermis has been removed. A surgical instrument for cutting skin grafts, called dermatome, is used to obtain samples of uniform shape and thickness. |
| <i>Stratum corneum</i> | The outermost layer of the epidermis. Consists of several layers of non-viable cells (typically 15–20), the outermost cells are lost by sloughing off. Varies in thickness with anatomical site. It presents the major barrier to dermal absorption. |
| Tape stripping | A procedure performed at the end of a dermal absorption study that involves the application of adhesive tape to the area of skin that was exposed to a chemical. An even (often predetermined) pressure is applied to the tape before it is removed, taking a layer of <i>stratum corneum</i> cells with it. The tape strip is then analysed to determine the amount of chemical that was present in the removed <i>stratum corneum</i> . The procedure is repeated to remove sequentially lower layers of the <i>stratum corneum</i> . |
| Transfer coefficient | The rate at which dislodgeable foliar residues can be transferred to a worker during a specified activity (expressed in terms of the area of contaminated foliage or fruit from which residues are transferred per hour). |
| $t_{0.5}$ | mean relative permeation into the receptor fluid occurring within half of the sampling period. |
| AOEL | acceptable operator exposure level |
| a.s. | active substances |
| BfR | Bundesinstitut für Risikobewertung |
| CPO | conditional predictive ordinate |
| DIC | deviance information criterion |
| ECETOC | European Centre for Ecotoxicology and Toxicology of Chemicals |
| ECPA | European Crop Protection Association |
| FDSP | finite dose skin permeation |
| GLP | Good Laboratory Practice |
| ML | marginal likelihood |
| MW | molecular weight |
| OECD | Organisation for Economic Co-operation and Development |
| PPP | plant protection product |
| PPR | EFSA Panel on Plant Protection Products and their Residues |
| QSAR | quantitative structure–activity relationship |
| RF | receptor fluid |
| SCCS | Scientific Committee on Consumer Safety |
| SCoFAH | Standing Committee on the Food Chain and Animal Health |
| WAIC | Watanabe-Akaike information criterion |
| WG | Working Group |
| WHO | World Health Organization |

Appendix A – Human *in vitro* dermal absorption data sets: new versions¹⁵

1. New ECPA data set

1.1. Data and methodology

European Crop Protection Association (ECPA) data pooled from 295 Good Laboratory Practice (GLP) and OECD 428-compliant *in vitro* human dermal absorption studies and collected into a data set (Aggarwal et al., 2014, 2015) were assessed by EFSA as described in the EFSA Scientific Report 'Assessment of new scientific studies on human *in vitro* dermal absorption' (EFSA, 2015). Individual values were not provided in the data set, in which mean values and standard deviations of dermal absorption parameters were reported at different levels of completeness. A new version of the ECPA data set was then created and submitted by ECPA, including individual replicate values from the human *in vitro* dermal absorption experiments. Moreover, additional and updated information was included in the new ECPA data set, such as: (i) added information: donor id, donor sex, donor age, number of replicates for each donor, replicate id; and (ii) updated information: skin region, % absorption at t0.5.¹⁶

The new data set, covering physicochemical properties for 152 agrochemical active substances (a.s.), 19 formulation types, tested at different concentrations, provided 5,180 records in total. Exposure and sampling time were 6–10 h and 24 h, respectively.

A plausibility check to verify the correctness of the data set from sources (study reports) was undertaken applying a protocol that, as a first step, aimed to identify relevant findings to be subsequently checked. Comparison was performed among data in the new ECPA data set (including individual replicates data) vs data in the first version of the ECPA data set (including mean values only). The relative deviation was calculated as the percentage ratio between mean value in the new data set and mean value in the first version of the data set. For acceptability purposes, a threshold for the relative variation was established (15%) so that only deviations above the threshold were considered initial findings and further evaluated in the plausibility check.

Both the parameters quantified in the dermal absorption experiment (non-absorbed dose, directly absorbed dose, whole skin dose, *stratum corneum* dose, tape strip 1 and 2 dose, overall recovery) and the calculated parameters (t0.5¹⁷ and absorbed dose) were evaluated. Thus, 6,080 pairs of means were compared (760 records checked for eight parameters).

1.2. Results

Application of the plausibility protocol identified 270 initial findings (relative variation between two mean values above 15%) out of 6,080 pair of mean values (4.4%).

For these findings, the plausibility check of data in the data set vs study reports allowed correction of the new ECPA data set for 56 findings,¹⁸ reducing the number of findings to 214 (3.5%).

Out of these 214 findings, 140¹⁹ were found to be accurate in the new ECPA data set. The remaining 74 relevant findings (1.2%) were due to missing data²⁰ and the t0.5 calculation.²¹

Individual values for tape strips 1 and 2 not found in the study reports but calculated from the relative amount in tape strip 1 and 2 were retrieved in the new data set.

In the end, from the analysis of normalised data found from the plausibility check, and considering the relevant number²² of recovery mean values outside EFSA acceptability (< 95%), it was decided to

¹⁵ The combined ECPA and BfR data sets used for the statistical analysis are provided as supporting information (XLS file).

¹⁶ t0.5: The mean relative permeation into the receptor fluid occurring within half of the sampling period.

¹⁷ t0.5 data were only partially reported in the first version of the ECPA data set and the plausibility check for this parameter was performed accordingly.

¹⁸ 34 mistakes in individual replicates data and/or mean values; 18 normalised data (low recovery) without justification; 3 not clear in the study reports (receptor fluid and 'swabbing' values, causing discrepancies for the calculation of directly absorbed dose; receptor fluid value was used); 1 mistake in the study reports.

¹⁹ 40 mistakes in the first version of the data set; 100 deviations due to whole skin dose calculation, accurate in the new data set.

²⁰ Reports for six studies in the ECPA data set were missing (not submitted to EFSA); 43 deviations were due to not reported tape strips 1 and 2 individual values in the study reports.

²¹ t0.5 calculation for different approaches (mean of % absorbed at 12 h relative to 24 h or as % of the mean absorbed at 12 h relative to 24 h).

²² 856 records out of 5,180.

apply the less stringent OECD (2011) requirement for the mean mass balance recovery, excluding low²³ (< 90%) and high²⁴ (> 110%) recovery mean values from the new data set and not normalise/use some data (as the justification was not provided). On the basis of this, final records in the new ECPA data set are 4972.

1.3. Conclusions

The plausibility check of the data in new ECPA data set (including individual replicate values) highlighted the high quality in data entry from the source (study report), discrepancies being found for only 1.2% of checked data. In particular, it was shown that the different approaches used to calculate t0.5 were leading to different outcomes and the need for clarification in the guidance was identified. In addition, it was noted that for a relevant number of records low and high mean mass balance recoveries were reported and it was decided to exclude from the statistical analysis records showing recovery mean values outside the OECD acceptability criteria.

2. New BfR data set

2.1. Data and methodology

The German Federal Institute for Risk Assessment (BfR) data set containing 480 records GLP and OECD 428-compliant *in vitro* human dermal absorption studies was assessed by EFSA, as described in the EFSA Scientific Report Assessment of new scientific studies on human *in vitro* dermal absorption (EFSA, 2015). Although mean values and standard deviations of dermal absorption parameters were reported, individual values were not provided in this data set. Also, t0.5 values were not included.

A new version of the data set was then created and submitted by BfR, including individual replicate values from human *in vitro* dermal absorption experiments. When available from the original reports, additional and updated information were included in the new data set, such as: (i) added information: donor id, donor sex, donor age, number of replicates for each donor, replicate id; and (ii) updated information: skin region, % absorption at t0.5.

As some overlap was noted between the original ECPA and BfR data sets, the updated BfR data set did not include those duplicates. Accordingly, the new data set, covering physicochemical properties for 94 agrochemical active substances, 23 formulation types and subtypes, tested in different formulations and at different concentrations, provided 2,273 individual records from 377 experiments in total. Exposure and sampling time were 6–24 h and 24 h, respectively. The structure of the new database was aligned by BfR with the EFSA requirements and reported, for example, separate values for total *stratum corneum* (all tape strips) and tapes strips 1 and 2, rather than only the *stratum corneum* excluding tape strips 1 and 2.

A quality control check was applied to the data set to verify the correctness of the data set from sources (study reports). This was undertaken applying a protocol that, as first step, aimed to identify relevant findings to be subsequently checked. A comparison was performed among data in the new BfR data set (including individual replicates data) vs data in the first version of the BfR data set (including mean and standard deviation values). The relative deviation was calculated as the percentage ratio between mean value in the new data set and mean value first version of the data set). For acceptability purposes, a threshold for the relative variation was established (15%) so that only deviations above the threshold were considered to be initial findings and further evaluated in the plausibility check. Both the parameters quantified in the dermal absorption experiment (directly absorbed dose; overall recovery) and the calculated parameters [absorbed dose (i.e. directly absorbed + whole skin – tape strips 1 and 2); t0.5] were evaluated. A total of 1,377 pairs of means were compared (377 experiments records checked for three or four parameters, as available in the first version).

A second step in the plausibility check of the individual record data was performed to address endpoints for which a direct comparison of means across replicates with the first version of the data set, as described in the EFSA Scientific Report issued in 2015, was not possible due to differences in the database structure, as described above. A set of 754 means was compared in this second step (377 experiments × 2 additional parameters). Thus, in total, 2,131 (1,377 + 754) pairs of means were checked.

²³ 141 out of 5,180 records were found < 90 and were removed from the new data set.

²⁴ 67 records out of 5,180 were found > 110 and were removed from the new data set.

2.2. Results

Application of the plausibility protocol identified 239²⁵ initial findings (relative variation between 2 mean values above 15%) out of $1,377 + 754 = 2,131$ pair of mean values (11%).

Out of these, 55²⁶ were traced to errors in the new data set. Correction of the new data set was performed using the original source data. This reduced the number of remaining findings to 184 (8.6%).

Another 26²⁷ findings were due to inappropriate rounding or erroneous pairing of means. These deviations were not considered relevant and no correction was triggered. Accordingly, the number of relevant finding was 158 (7.4%).

Out of these 158 findings, 130²⁸ were found to be accurate in the new BfR data set. The remaining 28 relevant findings (1.3%) were due to t0.5 calculation,²⁹ normalised data without justification,³⁰ and reporting of non-detects.³¹

As for the ECPA data set, it was decided to apply the less stringent OECD (2011) requirement for the mean mass balance recovery, excluding low³² (< 90%) and high³³ (> 110%) recovery mean values from the new data set. In addition, it was decided not to accept normalisation of the data for the purpose of this analysis, reducing the final number of records for individual values to 2,258.

2.3. Conclusions

The plausibility check of the data in new BfR data set (including individual replicate values) highlighted the high quality of data entry from the source (study report), discrepancies being found for only 1.3% of the checked data. In particular, different mathematical approaches used to calculate t0.5 leading to different outcomes were found, confirming the need for clarification in the guidance. Also, different approaches to the handling outliers (i.e. identification and potential exclusion) were noted when calculating mean values for experimental groups. More detailed guidance is provided on these issues to achieve better harmonisation in study evaluation.

²⁵ 156 from step 1 and 83 from step 2.

²⁶ 47 from step 1 and 8 from step 2.

²⁷ 11 from step 1 and 15 from step 2.

²⁸ 68 from step 1 and 62 from step 2.

²⁹ 16 findings.

³⁰ 8 findings were due to normalisation of mean data to overall recovery by the authors for two dose groups of one study.

³¹ 4 findings resulted from differences in reporting of non-detects (values below limit of quantification (LOQ)) between the data sets.

³² 260 records.

³³ 128 records.

Appendix B – Statistical analysis

1. Basis for the treatment of variability within the results

The values shown for the multiplication factor (k) in Table 1 (Section 5.3) are based on a mathematical model. Here, the model is described and its applicability evaluated; in particular, how it compares with the OECD approach which suggests using the highest replicate in risk assessment when the variability is high (OECD, 2011).

Suppose that a sample of n values x_1, \dots, x_n is drawn from a normally distributed population. Then, the standard 95% confidence interval for the population mean is well known:

$$\bar{x} \pm t^* \frac{s}{\sqrt{n}} \quad (\text{B1})$$

where \bar{x} is the sample mean, s is the sample standard deviation and t^* is the value which is exceeded with probability of 2.5% by the Student's t distribution having $n - 1$ degrees of freedom. In Table 1, $k = t^* / \sqrt{n}$.

The upper 97.5% confidence limit is the upper end of the 95% confidence interval and underestimates the population mean for 2.5% of samples. This holds true for any n . By contrast, when $n = 4$, the sample maximum underestimates the population mean for 6.3% of samples from a normal distribution and this percentage decreases as n increases. When $n = 8$, it becomes 0.4%. So, the degree of conservatism obtained by using the maximum changes greatly with the sample size. Moreover, it is known mathematically that the most statistically efficient way to estimate an upper limit for the population mean is to take the upper limit confidence limit. Thus, not only does the overall level of conservatism change with n if the sample maximum is used, but also the amount of conservatism varies more between samples if the maximum is used.

Therefore, it is sensible to seek an alternative to using the sample maximum. However, absorption replicates are not normally distributed, even within a single study. They tend to be skewed quite substantially: for the data set (ECPA + BfR) described in Appendix A, 77% of samples have positive skew, the mean skewness is 0.5 and the standard deviation of skewness is 0.7. One solution to this problem is to transform data before making statistical calculations. In this case, the logit transformation:

$$\ln(x/(1 - x))$$

has several benefits: the average skewness is close to 0 for the transformed data; there is little evidence that skewness depends on the amount of absorption; and the variability of the transformed data is stabilised so that it no longer depends strongly on the level of absorption (homogeneity). With respect to these criteria, the logit transformation was found to be superior to either the probit or arcsine transformations which are common alternatives. The logit transformation is essentially indistinguishable from log transformation when the fraction absorbed is small, but stretches the scale out better for higher levels of absorption.

The above argument that the upper confidence limit is preferable to the sample maximum for normally distributed data is much more directly applicable to logit-transformed absorption data than to raw (untransformed) absorption data since normal distributions are symmetric. Note that, due to low power, there is little point in testing for normality for the small samples sizes that are usual in absorption studies. To apply the upper confidence limit to transformed data, one would first calculate the logit of each replicate, then calculate the upper confidence limit (UCL) from the transformed data and finally undo the logit transformation by calculating:

$$\exp(\text{UCL}) / (1 + \exp(\text{UCL})).$$

The resulting value would be a 97.5% upper confidence limit for the population median fraction absorbed. This is because the mean and median are the same for symmetric distributions and the median of logit transformed values is the same as the logit of the median of untransformed values.

However, logit transformation requires more effort to apply than simply calculating the upper confidence limit directly using the raw data. As a practical compromise, it is suggested to apply the

upper confidence limit calculation to the untransformed absorption values without using any transformations. An empirical investigation follows of the performance of the method when applied to the data set (ECPA + BfR) described in Appendix A.

Figure B.1 shows box plots, by sample size n , of the ratio of the upper confidence limit obtained using the logit approach to the upper confidence limit obtained by applying equation 1 to the raw data. It shows that the logit approach is typically more conservative for $n = 4$, slightly more conservative for $n = 5$, about the same for $n = 6$ and slightly less conservative for $n = 7$ and $n = 8$. There is not much difference overall for $n \geq 5$. There are quite large differences in outcome for some samples but this reflects the fact that small sample sizes generally lead to variable outcomes for any statistic.

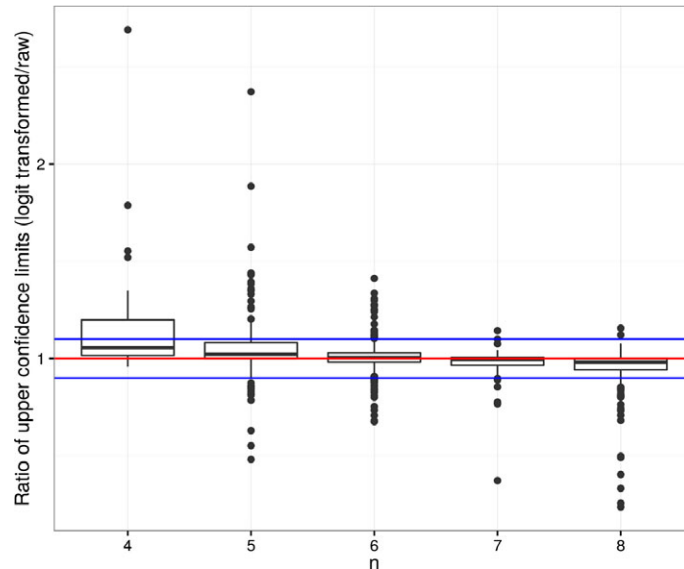


Figure B.1: Box plots, by sample size n , of the ratio of logit-transform based upper confidence limit to raw data upper confidence limit. Red line highlights where the limits are equal and blue lines where the ratio is between 0.9 and 1.1.

Figure B.2 shows box plots, by sample size n , of the ratio of the upper confidence limit obtained using the raw data to the maximum of the sample. For $n = 4$, it shows that the upper confidence limit is more conservative than the maximum even though it is less conservative than when the logit transformation is used. So, for $n = 4$, the upper confidence limit is a step in the right direction. For $n \geq 5$, the upper confidence limit is, as desired, less conservative than the maximum and is not very much less conservative than when the logit transformation is used.

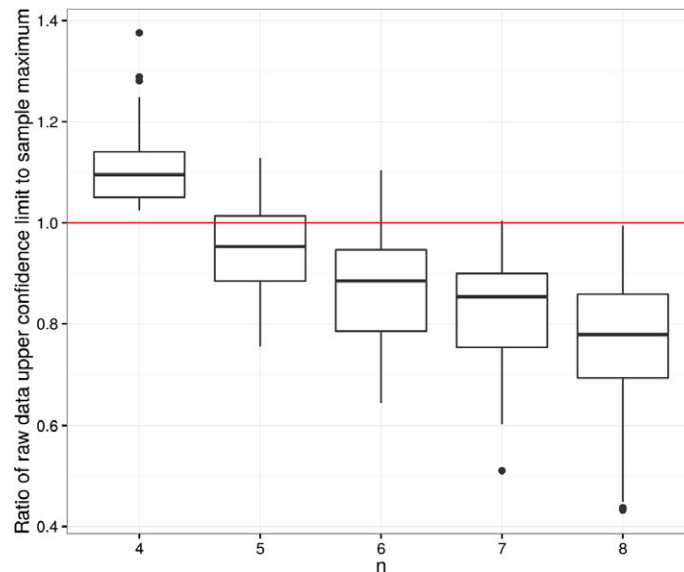


Figure B.2: Box plots, by sample size n , of the ratio of raw data upper confidence limit to the sample maximum. Red line highlights where the limits are equal

Finally, the upper confidence limit calculation assumes that the sampled values are independent. When the number of donors is less than the number of samples, there is some correlation between samples from the same donor. In principle, the values for k in the Table 1 in Section 5.3 should be increased to allow for this correlation. The typically small sample size means that the estimate of this correlation is extremely variable between samples; on average, it was found to be 0.3 and this could be considered a sensible value to use. On that basis, Monte Carlo simulation was used to calculate the value of k for each value of n in the Table 1 in section 5.3, making the assumption that there were two donors for $n < 5$, three donors for $n = 5$ and four donors for $n > 5$, and that replicates were as evenly balanced across donors as possible. In each case, the increase in k compared to the value in the Table 1 (Section 5.3) was less than 20%. Empirically, using untransformed data, the upper confidence limit would increase by less than 10% (e.g. from 1% absorption to 1.1% absorption) for 90% of samples and never by more than 15%. Since the use of the maximum in any case makes no allowance for correlations, the values of k in the table were left unchanged.

2. Pro-rata extrapolation for untested dilutions

Figure B.3 shows data from studies in the data set (ECPA + BfR) described in Appendix A, where the same active substance was tested at more than one dilution. Replicates were averaged on a logit scale and back-transformed to the original scale as described in Section 1 of Appendix B. It also shows lines highlighting pro-rata extrapolation and the proposal of Aggarwal et al. (2014, 2015) to make no extrapolation for less than twofold dilution and to cap pro-rata extrapolation at a maximum of fivefold increase in absorption. Points are coloured light blue when pro-rata extrapolation would predict absorption in excess of 30%, the situation for which Aggarwal et al. (2014, 2015) further propose capping absorption at 30%.

There were, in total, 8 data points above the red line highlighting the EFSA pro rata approach for extrapolation (partially not resolved in Figure B.3). For these data points, mean dermal absorption percentages increased by a factor that was higher than the quotient of the concentrations tested. For seven of these eight cases, the ratio between increase in mean dermal absorption and decrease in concentration tested was between 1.02 and 1.26, rather than the worst-case of 1.00 assumed in the EFSA pro-rata approach. However, this level of deviation is within the limits of variability observed for the test method and would not invalidate the approach. For the remaining case, the mean dermal absorption increase 2.53-fold as strong as greater than would be expected when applying the EFSA pro-rata approach. However, re-inspection of the data showed that automated data analysis lead to inappropriate comparison of a dilution of a product with another dilution of the same product to which additional surfactant was added while the difference in active substance concentration was minimal. Accordingly, the larger than expected increase in dermal absorption could be attributed to the added co-formulant.

As depicted in Figure B.3, several data points would be underpredicted by the extrapolation approach proposed by Aggarwal et al. (2014, 2015) and the magnitude of underprediction can be significant (note logarithmic scale in Figure B.3).

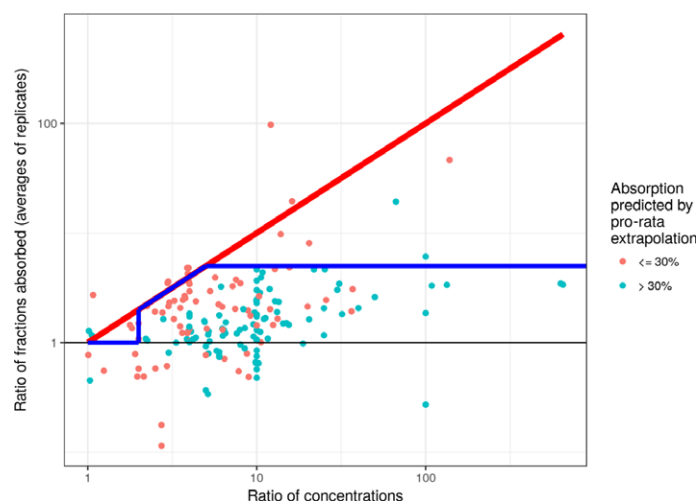


Figure B.3: Exploration of pro-rata extrapolation for dilutions. Each point corresponds to a study with more than one dilution of the same active substance. The horizontal axis shows the ratio of the concentrations for the two dilutions. The vertical axis shows the ratio of the fraction absorbed (average of replicates) for the lower concentration to the fraction absorbed at the higher concentration (average of replicates). Both axes are logarithmic. The thick red line corresponds to pro-rata extrapolation and the thick blue line corresponds to Aggarwal et al. (2014, 2015); points coloured light blue are cases where pro-rata extrapolation would predict absorption in excess of 30%, i.e. where step 3 in the extrapolation scheme proposed in section 4.7 of Aggarwal et al. (2014) would apply

3. Statistical modelling to support the setting of default values

For the purpose of calculations to support the setting of default values, two different statistical modelling approaches were tried.³⁴ Both were based on the data set (ECPA + BfR) described in Appendix A and explored how variation in dermal absorption could be explained by physicochemical properties of the active substance, by the type of formulation used, by properties of the skin sample exposed and by experimental conditions (see Table B.1).

Table B.1: Evaluated variables and corresponding parameters in the data set (ECPA + BfR)

| | |
|--|---|
| Physicochemical properties (active substances) and formulation (product) | Active substance code, MW, log P_{ow} , water solubility, formulation type, concentration tested, concentration status (concentrate/dilution) |
| Skin sample | Skin type, sex, age, donor, skin source, skin region |
| Experimental conditions | Exposure duration, chamber type, mass balance recovery, receptor medium |

MW: molecular weight.

The physicochemical variables included log-partition coefficient (log P_{ow}), molecular weight (MW) and water solubility. The active substance name was also included as a categorical variable in some models to act as a proxy for differences between substances other than the provided physicochemical variables. Because the data set (ECPA + BfR) included many different formulation types, they were merged into four categories: organic solvent, water-based, solid and 'other'. Grouping of formulations types is based on information about the chemical composition of the tested product, the information on the phase in which the active substance is dissolved or emulsified/suspended and the expectable impact on dermal absorption (see Table B.2). Skin sample variables included skin type, sex and age. The data set (ECPA + BfR) also includes skin source, skin region and donor id, which were disregarded

³⁴ All R codes used for the analysis are provided as supporting information (HTML file).

in most analyses since they were missing from many data records. The experimental condition variables included log-concentration (mass and molar) of the active substance, duration of exposure, chamber type, recovery and receptor medium.

Because the fraction of chemical absorbed must lie between 0 and 1, a standard regression is not appropriate as it assumes that random variations are normally distributed. One statistical modelling approach addressed this by using beta regression and the other by applying standard and mixed model (also known as random-effects or multilevel) regression methods to the logit of the fraction absorbed.

Table B.2: Formulations categories. Grouping of formulation types in four categories based on the tested product types in the data set (ECPA + BfR): (1) Primarily organic solvent-based, (2) Primarily water-based/dispersed, (3) Solid, (4) Other

| | Code | Description | Number of studies | Details and justification for grouping ^(a) |
|---|-------|----------------------------------|-------------------|---|
| 1 | EC | Emulsifiable concentrate | 90 | The active substance is dissolved in suitable organic solvents, together with any other necessary formulants. It should be in the form of a stable homogeneous liquid, free from visible suspended matter and sediment, to be applied as an emulsion after dilution in water |
| | DC | Dispersible concentrate | 1 | The active substance is dissolved in suitable organic solvents, together with any other necessary formulants. It should be in the form of a stable homogeneous liquid, free from visible suspended matter and sediment, to be applied as a dispersion after dilution in water |
| | EW | Emulsion, oil in water | 15 | A stable emulsion of active substance(s) in an aqueous phase, intended for dilution with water before use. The active substance is normally a liquid and forms the dispersed oil phase, but it is also possible to emulsify a solid or liquid active ingredient dissolved in a water-immiscible solvent. EW are emulsions with organic solvents as the inner phase in which the active substance is dissolved |
| | SE | Suspo-emulsion | 29 | Multiphase formulation whereby an emulsified active substance is combined with active suspended in water. SEs are emulsions/suspensions with an organic solvents(s) as a phase in which the active substance is dissolved |
| | OL/OF | Oil-miscible liquids | 1 | A solution of the active substance, together with any other necessary formulants, in an organic solvent; no water is contained. It should be free of visible suspended matter and sediment, intended for dilution with organic liquid before use |
| | OD | Oil-based suspension concentrate | 18 | An oil dispersion is a stable suspension of active substance(s) in an organic fluid, which may contain other dissolved active substance(s); no water is contained. It is intended for dilution with water before use |
| | ES | Emulsion for seed treatment | 1 | A water-based emulsion containing the active substance, together with large amounts of organic solvents and any necessary formulants including colouring matter. It should be easy to homogenise, and suitable for dilution with water if necessary, application to the seed either directly or after dilution. ES are emulsions with organic solvents as inner phase in which the active substance is dissolved |
| | ME | Microemulsion | 3 | A mixture of water, water-insoluble and water-soluble components forming a visually homogeneous, transparent liquid. One or more active substances may be present in either the aqueous phase, the non-aqueous phase, or in both phases. A variety of microemulsion formulations may be prepared in which the aqueous phase can be considered the dispersed phase, the continuous phase or, alternatively, where the two phases are considered bicontinuous. In all cases, microemulsions will disperse into water to form either conventional emulsions or dilute microemulsions |

| | Code | Description | Number of studies | Details and justification for grouping ^(a) |
|---|--------|---|-------------------|--|
| 2 | SL | Soluble concentrate | 21 | Water-based formulation in which a salt of pesticide acid is dissolved in water, together with any other necessary formulants. It should be in the form of a clear or opalescent liquid, free from visible suspended matter and sediment, to be applied as a true solution of the active substance in water |
| | SC | Suspension concentrate | 121 | A stable suspension of active substance(s) in an aqueous continuous phase, intended for dilution with water before use |
| | FS | Flowable concentrate for seed treatment | 18 | A suspension of fine particles of the active substance in an aqueous phase together with suitable formulants, including colouring matter. After gentle stirring or shaking, the material shall be homogeneous and suitable for further dilution with water if necessary |
| | FL | Flowable | 1 | As described for the product, it is a SC formulation |
| 3 | WP | Wettable powder | 12 | A homogeneous mixture of the active substance(s) together with filler(s) and any other necessary formulants. It should be in the form of a fine powder free from visible extraneous matter and hard lumps |
| | WG/WDG | Water-dispersible granules | 57 | Intended for application after disintegration and dispersion in water by conventional spraying equipment |
| | SG | Water-soluble granules | 5 | Granules containing the active substance, and, if required, suitable carriers and/or necessary formulants. It shall be homogeneous, free from visible extraneous matter and/or hard lumps, free flowing, and nearly dust-free or essentially non-dusty. The active substance should be soluble in water |
| | SP | Water-soluble powder | 2 | A homogeneous mixture of the active substance, together with any necessary formulants. It should be in the form of a powder to be applied as a true solution of the active substance after solution in water, but which may contain insoluble inert ingredients |
| | DS | Powder for dry seed treatment | 1 | A powder for application in the dry state directly to the seed; a homogeneous mixture of the active substance together with suitable fillers and any other necessary formulants including colouring matter. It should be in the form of a fine free-flowing powder, free from visible extraneous matter and hard lumps |

| | Code | Description | Number of studies | Details and justification for grouping ^(a) |
|-------------------------------------|---------------------------------|--|-------------------|--|
| 4 | CB | Bait concentrate | 1 | A solid or liquid intended for dilution before use as a bait. Sugar or other food ingredients or components like talcum are often the main compounds |
| | CS | Capsule suspension | 6 | A stable suspension of microencapsulated active substance in an aqueous continuous phase, intended for dilution with water before use |
| | GEL/GD | Gel for direct application | 1 | A gel-like preparation, intended to be applied undiluted. A gel for direct application consists of one or more active substances, a structuring agent and other formulants if appropriate. The active substance is homogenised in suitable solvents, together with any other necessary formulants. It should be in the form of a clear or opalescent gel, free from visible suspended matter and sediment. These formulations are different from organic or water-based formulations |
| | RB/Pellets/Wax block/Pasta bait | Bait (ready for use; paste, wax bloc, pasta bait included) | 6 | A formulation designated to attract and be eaten by the target pests. Wax blocs or paste baits are included. For example, in the case of a rodenticide, it is a solid bait and is based on grains, cereals and/or large amounts of wax. RB differs from other solid formulations |
| | ZC | CS and SC mixture | 3 | A mixed formulation of CS and SC and is a stable suspension of microcapsules and solid fine particles, each of which contains one or more active substances. The formulation is intended for dilution into water prior to spray application |
| | PS | Seed coated with a pesticide | 1 | Seed coated with a pesticide. PS formulations probably contain only the active substance, colour and carrier formulants |
| | AI | Experimental solution of active substance in solvent | 4 | The vehicle can be organic or water-based. Because of the absence of other co-formulants, these solutions are not comparable with organic- or water-based formulations |
| Excluded from statistical analysis: | | | | |
| | XX/NA | | 6 | No information on formulation type available |

Numbers of studies are reported for each formulation type in the data set (ECPA + BfR).

(a): Based on the FAO/WHO, 2016.

3.1. Beta regression modelling

In this part of the analysis, a family of Beta regression models was used, a type of regression model tailored to analyse data with a continuous response variable lying in (0,1). The response variable is assumed to be Beta distributed, which in the usual parameterisation has probability density function

$$f(y) = \frac{\Gamma(a+b)}{\Gamma(a)\Gamma(b)} y^{a-1} (1-y)^{b-1}$$

for $y \in (0, 1)$ with parameters $a, b, > 0$. Because the expectation μ relates to parameters a and b through the equation $\mu = a/(a+b)$, this parameterisation is not the most convenient for regression analysis and the alternative parameterisation below is used in the Beta regression framework:

$$f(y) = \frac{\Gamma(\phi)}{\Gamma(\mu\phi)\Gamma((1-\mu)\phi)} y^{\mu\phi-1} (1-y)^{(1-\mu)\phi-1}$$

with $\mu \in (0, 1)$ (mean) and $\phi > 0$ (precision parameter), which corresponds to the change of variable $\mu = a/(a+b)$ and $\phi = a+b$.

The Beta regression model considered in the present analysis assumes that the expectation μ of the response y relates to a vector of explanatory variables $\mathbf{x} = (x_1, \dots, x_p)^t$ through the logistic

function, namely $\mu = 1/(1 + \exp(-\mathbf{x}^t\boldsymbol{\beta}))$ where $\boldsymbol{\beta}$ is a vector of unknown regression parameters. This means that $\mathbf{x}^t\boldsymbol{\beta}$ is the logit of μ .

The explanatory variables considered were those listed in Table B.1 with the exception of skin donor, source and region which are missing from many records. For the modelling for which results are reported in detail in the rest of Section 3.1 of Appendix B, concentration tested was included using the molar concentration. Moreover, formulation type and concentration status (concentrate/dilution) were included via a single synthetic categorical variable (named FormTypeConcDil in Tables B.3 and B.4 below) combining the grouped formulation described earlier (organic solvent, water-based, solid and 'other') with the concentration status. The five levels of that categorical variable were: organic solvent formulation tested as concentrate, water-based formulation tested as concentrate, solid formulation tested as concentrate, other formulation tested as concentrate and any formulation tested as dilution. Active substance is a factor (categorical variable) with 238 levels and stands as a surrogate for non-observed structural information about the various active substances. It was treated as a random effect, making the model a mixed model. Receptor medium is also a factor which after aggregation of the initial 180 levels has 14 levels. It was also treated as a random effect. The analysis was performed on those records in the data set for which the recorded absorption was greater than 0% and less than 100%.

Each variable being included or not in a submodel, the list of 13 explanatory variables gives rise to $2^{13} = 8192$ submodels. For each submodel, parameters were estimated and several model fit criteria were computed: the Deviance Information Criterion (DIC), the Marginal Likelihood (ML), the Watanabe-Akaike information criterion (WAIC) and the Conditional Predictive Ordinate (CPO). Computing model fit criteria for those 8,192 submodels allows quantification of how each explanatory variable contributes to explaining variation in dermal absorption while accounting for potential confounding effects. Computations were made using the R package INLA (Rue et al., 2009).

3.1.1. Results

A strong congruence in the way the various model fit criteria ranked the 8192 submodels was observed, in particular among DIC, WAIC and CPO. Therefore, it was decided to focus on DIC for subsequent analyses. DIC was used to rank the various submodels and for each k from 1 to 13, the best submodel containing exactly k variables was identified. The results are given in Table B.3.

Table B.3: List of best k -variable models in terms of Deviance Information Criterion.

| k (number of explanatory variables) | Best submodel | -DIC |
|--|--|-------------|
| 1 | FormTypeConcDil | 22,021.54 |
| 2 | logMolConc + AScode | 25,124.00 |
| 3 | logMolConc + ReceptMedium + AScode | 25,438.65 |
| 4 | logMolConc + Duration + ReceptMedium + AScode | 25,612.36 |
| 5 | FormTypeConcDil + logMolConc + Duration + ReceptMedium + AScode | 25,723.63 |
| 6 | FormTypeConcDil + logMolConc + SkinType + Duration + ReceptMedium + AScode | 25,809.91 |
| 7 | WaterSolub + FormTypeConcDil + logMolConc + SkinType + Duration + ReceptMedium + AScode | 25,844.12 |
| 8 | WaterSolub + FormTypeConcDil + logMolConc + SkinType + Duration + ChamberType + ReceptMedium + AScode | 25,873.17 |
| 9 | WaterSolub + FormTypeConcDil + logMolConc + SkinType + Age + Duration + ChamberType + ReceptMedium + AScode | 25,901.05 |
| 10 | logMW + WaterSolub + FormTypeConcDil + logMolConc + SkinType + Age + Duration + ChamberType + ReceptMedium + AScode | 25,925.38 |
| 11 | logMW + WaterSolub + FormTypeConcDil + logMolConc + SkinType + Age + Sex + Duration + ChamberType + ReceptMedium + AScode | 25,942.34 |
| 12 | logMW + WaterSolub + FormTypeConcDil + logMolConc + SkinType + Age + Sex + Duration + ChamberType + ReceptMedium + AScode + Recovery | 25,950.67 |

| k (number of explanatory variables) | Best submodel | –DIC |
|-------------------------------------|---|-----------|
| 13 | logMW + logP _{ow} + WaterSolub + FormTypeConcDil + logMolConc + SkinType + Age + Sex + Duration + ChamberType + ReceptMedium + AScore + Recovery | 25,946.93 |

FormTypeConcDil is the five level categorical variable combining formulation type and concentration status defined above, logMolConc = 'log molar concentration', AScore = 'active substance code', ReceptMedium = 'receptor medium', Duration = 'exposure duration', SkinType = 'skin type', WaterSolub = 'water solubility', ChamberType = 'chamber type', logMW = 'logarithm of molecular weight', Recovery = 'mass balance recovery', logP_{ow} = 'logarithm of P_{ow}'.

This ranking is helpful to understand which variables explain best variation in dermal absorption. The most striking change in DIC is from k = 1 to k = 2 when the active substance code appears for the first time and it should be noted that it appears in all subsequent models. The models in Table B.3 are only a small subset of all models fitted and there are often other models with the same number of explanatory variables which exhibit nearly as good performance.

All models from k = 2 onwards include active substance code as an explanatory variable and all from k = 3 onwards include receptor medium. For the active substance random effect term, the model coefficients are substance-specific and can be estimated only for substances present in the data set (ECPA + BfR). A model based on the active substance random effect term is, therefore, unable to provide a substance-specific prediction of dermal absorption for a substance not in the data set (ECPA + BfR) although it is possible to make a generic prediction that should cover a specified percentage of substances. Consequently, a model which includes the active substance code or the receptor medium poses some challenges for designing default values since the random effects aspect of either variable must be addressed. This is technically difficult to do for the Beta regression model fitted using INLA and this aspect is therefore addressed later in Section 3.2 of Appendix B using random effects logit regression models.

In a second step, for each k from 1 to 11, the best submodel containing exactly k variables, excluding active substance code and receptor medium, was identified. These submodels are given in Table B.4. The synthetic variable FormTypeConcDil combining information about the formulation type and the concentration status appears in all models in Table B.4 and is an important variable for predicting dermal absorption. Other variables, for example exposure duration and skin type, might be seen as playing an important role. However, the improvement in DIC value when these variables are included, or even when all 11 variables are included, is modest compared to the improvement when active substance code is included in the second model in Table B.3. This is evidence of confounding between active substance code and other variables and this confounding is discussed further in Section 3.2 of Appendix B.

Table B.4: List of best k–variable, excluding active substance code and receptor medium, models in terms of Deviance Information Criterion. For the meaning of abbreviations, see the caption to Table B.3.

| k (number of explanatory variables) | Best submodel without AScore and Receptor Medium | –DIC |
|-------------------------------------|--|-----------|
| 1 | FormTypeConcDil | 22,021.54 |
| 2 | FormTypeConcDil + Duration | 22,327.99 |
| 3 | FormTypeConcDil + SkinType + Duration | 22,435.64 |
| 4 | FormTypeConcDil + SkinType + Age + Duration | 22,534.35 |
| 5 | FormTypeConcDil + logMolConc + SkinType + Age + Duration | 22,616.08 |
| 6 | logMW + logP _{ow} + FormTypeConcDil + logMolConc + SkinType + Duration | 22,662.22 |
| 7 | logMW + logP _{ow} + FormTypeConcDil + logMolConc + SkinType + Age + Duration | 22,769.96 |
| 8 | logMW + logP _{ow} + FormTypeConcDil + logMolConc + SkinType + Age + Duration + ChamberType | 22,791.25 |
| 9 | logMW + logP _{ow} + WaterSolub + FormTypeConcDil + logMolConc + SkinType + Age + Duration + ChamberType | 22,802.63 |

| k (number of explanatory variables) | Best submodel without AScore and Receptor Medium | –DIC |
|-------------------------------------|---|-----------|
| 10 | logMW + logP _{ow} + WaterSolub + FormTypeConcDil + logMolConc + SkinType + Age + Duration + ChamberType + Recovery | 22,801.07 |
| 11 | logMW + logP _{ow} + WaterSolub + FormTypeConcDil + logMolConc + SkinType + Age + Sex + Duration + ChamberType + Recovery | 22,797.15 |

Diagnostic analysis suggested that the Beta regression models provide a fit of modest quality. Consequently, no further use was made of Beta regression models in arriving at the recommendations for default values provided in Section 5 of Appendix B. However, the knowledge gained from the modelling as described above helped to define the categories used for defining default values.

3.2. Logit regression modelling

The logit function transforms values between 0 and 1 to the whole real line. It is widely used in statistical analysis of proportions and probabilities (e.g. the logistic regression method). Here, the logit function was applied to the fraction absorbed and the resulting variable was used as the response for standard regression modelling. As described in Section 1 of Appendix B, the logit transformation applied to the fraction absorbed provides a variable that is less skewed and for which variability is more homogeneous. However, approximately 2% of the records in the data set (ECPA + BfR) have zero absorption and they would have to be excluded if the logit transformation was not adapted. For this reason, all fraction-absorbed values were 'shrunk' slightly towards 0.5 by first calculating $0.5 + 0.9995(x - 0.5)$ before applying the logit transformation. The shrinkage constant 0.9995 was arrived at by experimenting to find a number that makes the zero absorption cases fit naturally on the tail of the distribution of resulting logit values.

The range of models considered was influenced by the results of the beta regression modelling in Section 3.1 of Appendix B and a smaller range of models was explored. The conclusions were essentially the same: of the physicochemical, formulation category, skin and experimental condition variables, only concentration status (concentrate/dilution) and the formulation grouping into categories were found to explain a substantial amount of variation of absorption.

Figure B.4 shows the dependence of fraction absorbed on concentration, using a logit scale for fraction absorbed and a logarithmic scale for concentration. There is a clear difference between dilutions and concentrates and any further dependence on concentration is weak. There is overlap in terms of concentration between the dilutions and concentrates and this precludes establishing a concentration threshold to distinguish dilutions from concentrates. Figure B.5 shows, separately for dilutions and concentrates, the dependence of fraction absorbed on the formulation category.

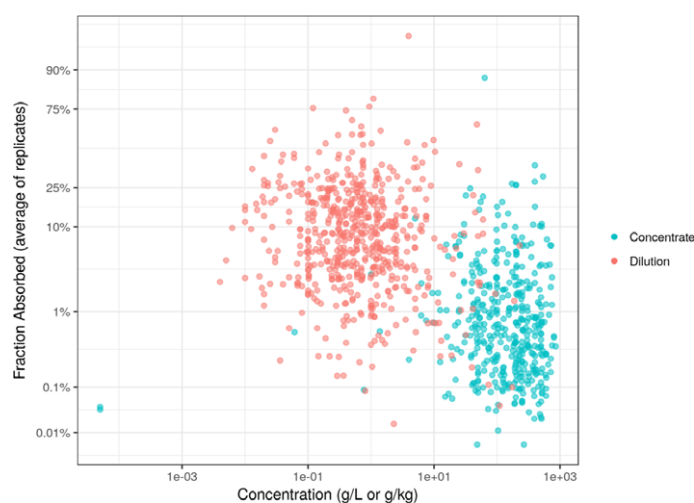


Figure B.4: Dependence of fraction absorbed (logit scale) on concentration (logarithmic scale)

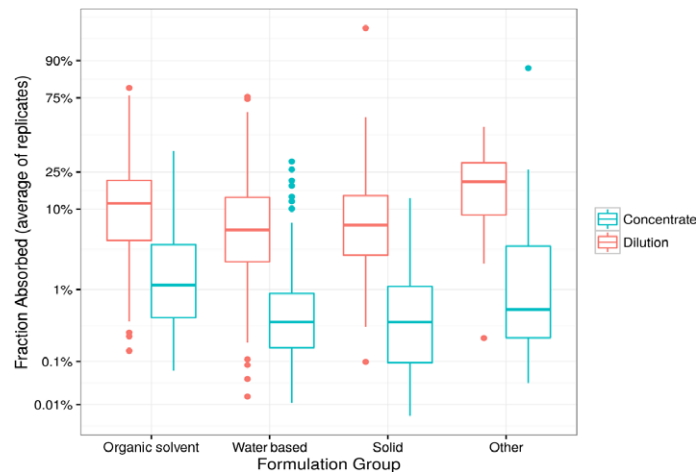


Figure B.5: Dependence of fraction absorbed (logit scale) on formulation category and on concentration status (dilution/concentrate)

An important tool in standard regression modelling (e.g. Draper and Smith, 1998) is sequential analysis of variance which tries to show how much explanatory variables account for variation in the response. Several analyses of variance were carried out to establish as far as possible the sequence of sources of variation which best accounts for variation of the response. The preferred analysis first includes the formulation category and concentration status (dilution/concentrate) variables and then adds in turn:

- the active substance categorical variable and per-substance difference between dilution and concentrate;
- the study identifier and within-study variation between different concentrations and detailed formulations for the same substance;
- donor id within study.

Other analyses not presented in detail here considered the role of explanatory variables identified as potentially important in Tables B.3 and B.4: exposure duration, receptor medium and skin type. A categorical variable indicating the data source (ECPA or BfR) from which a measurement came was also considered. It was found that while each of these variables could explain a substantial amount of variation if included in the model prior to the active substance variable, it explained relatively little variation if included after the active substance variable. This is evidence of substantial confounding between these other variables and the active substance variable. Since none of these other variables is a property of the active substance or of its formulation they were omitted from subsequent modelling. As a result, variation which is explained by them, but not by active substance code, is effectively included in the variation explained by terms included in the second bullet above.

3.2.1. Results

The results are shown in Tables B.5 and B.6. Table B.5 uses the full data set (ECPA + BfR) and therefore omits donor id. Table B.6 is based on those records in which donor id was recorded. Despite being based on a subset of the data, it reaches very similar conclusions about the proportions of variance explained by the various sources and provides additional information about the decomposition of replication variation into between- and within-donor components.

On the basis of Table B.5, a random-effects multiple regression model (e.g. Pinheiro and Bates, 2002) was fitted, taking the logit of (shrunk) fraction-absorbed as the response variable and estimating fixed effects for the formulation category and concentrate/dilution and random effects for each of the other sources of variation in Table B.5. The model was fitted in R (R Core Team, 2016) using the Bayesian MCMCglmm package (Hadfield, 2010) and the results checked for consistency with more traditional restricted maximum likelihood fitting using the lme4 package (Bates et al., 2015). An advantage of the Bayesian approach is that uncertainty about parameter estimates is easily accessed and used to obtain uncertainty about quantities derived from the parameters (see Section 5.2 of Appendix B).

Table B.5: Sequential analysis of variance for the full data set (ECPA + BfR)

| Source block | Source of variation | Degrees of freedom | Sum of squares | Mean square | % variation |
|--------------|--|--------------------|----------------|-------------|-------------|
| 1 | Dilution/concentrate | 1 | 9,119 | 9,119 | 33 |
| | Formulation category | 3 | 849 | 283 | 3 |
| 2 | Active substance | 233 | 7,813 | 34 | 28 |
| | Active substance specific dilution/concentrate | 213 | 2,097 | 9.8 | 7 |
| 3 | Study | 194 | 2,422 | 12 | 9 |
| | Concentrations and formulations for same active substance within study | 342 | 1,526 | 4.5 | 5 |
| 4 | Replicates | 5778 | 4,153 | 0.72 | 15 |

Table B.6: Sequential analysis for the reduced data set consisting of records which include donor id

| Source block | Source of variation | Degrees of freedom | Sum of squares | Mean square | % variation |
|--------------|--|--------------------|----------------|-------------|-------------|
| 1 | Dilution/concentrate | 1 | 7,848 | 7,848 | 32 |
| | Formulation category | 3 | 836 | 279 | 3 |
| 2 | Active substance | 205 | 6,741 | 33 | 28 |
| | Active substance specific dilution/concentrate | 183 | 1,732 | 9.5 | 7 |
| 3 | Study | 162 | 2,137 | 13 | 9 |
| | Concentrations and formulations for same active substance within study | 307 | 1,325 | 4.3 | 5 |
| 4 | Replicates: between donors | 2362 | 2,472 | 1.0 | 10 |
| | Replicates: within donor | 2602 | 1,252 | 0.5 | 5 |

4. The role of P_{ow} and MW

The EFSA PPR Panel guidance on dermal absorption issued in 2012 states that if $\log P_{ow} < -1$ or > 4 and $MW > 500$ a default dermal absorption value of 10% may be applied (EFSA PPR Panel, 2012) but does not provide detailed reasoning to support the default value. This focusses attention on how absorption depends on MW and P_{ow} .

P_{ow} and MW are continuous quantitative variables and there is no well identified knowledge supporting the idea that the biochemical process of dermal absorption undergoes a transition beyond the cut-off $\log P_{ow} < -1$ or > 4 and $MW > 500$, with specific consequences for dermal absorption values. Therefore, for the statistical modelling, P_{ow} and MW were treated as continuous variables having potentially a continuous effect on dermal absorption.

Figure B.6 shows the relation between fraction absorbed and $\log P_{ow}$. Figure B.7 shows the relation between fraction absorbed and MW. Neither figure shows any strong indication of a predictive relationship. A similar lack of a strong relationship was found when figures were produced separately for each combination of formulation category and concentration status. This kind of graphical investigation could be misleading due to possible confounding effects. However, the finding is consistent with the statistical modelling results which did not show a big role for either explanatory variable.

For records in this category, median and mean fraction absorbed are, respectively, 3.1% and 5.7%. The 75th, 95th and 99th percentiles are 18%, 31% and 34%. These are not strikingly different from summaries for the whole data set, especially given that the distribution of formulation category and concentration status is not the same.

Therefore, no specific default values are proposed in Section 5 of Appendix B and Section 6.1 for this category of active substances.

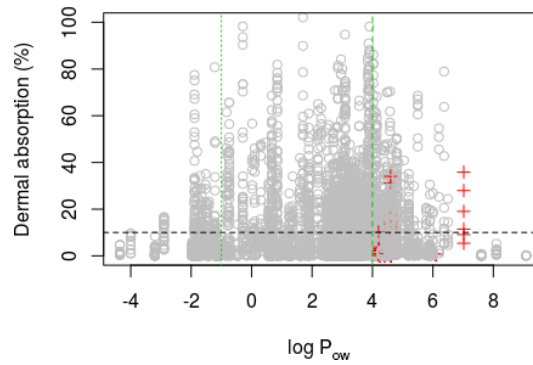


Figure B.6: Scatter plot of dermal absorption vs $\log P_{ow}$. Vertical green lines depict cut off at $\log P_{ow} < -1$ and > 4 . Horizontal black line depicts the 10% value. Red crosses denote records for which $\log P_{ow} < -1$ or > 4 and $MW > 500$

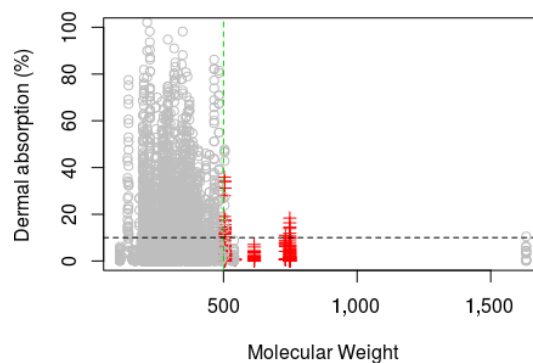


Figure B.7: Scatter plot of dermal absorption vs molecular weight. Vertical green line depicts cut off at $MW > 500$. Horizontal black line depicts the 10% value. Red crosses denote records for which $\log P_{ow} < -1$ or > 4 and $MW > 500$

5. Calculations intended to support setting of default values

Two approaches are provided to calculations intended to support setting of default values: empirical percentiles and model-based percentiles. Results are presented for: eight categories based on four formulation categories and distinguishing dilutions from concentrate products, and four categories based on combining the formulation categories to make two groups and again distinguishing dilutions from concentrate products.

5.1. Empirical percentiles

Empirical percentiles are shown in Tables B.7 and B.8. The values shown are for the 95th, 97.5th and 99th percentiles. Because the values shown are estimates obtained from a particular data set, they are subject to uncertainty. Therefore, the upper bound of a one-sided $\geq 95\%$ confidence interval is also shown for each estimated percentile. The confidence interval calculation is based on the assumption that the data are a random sample from a homogeneous population. This assumption does not hold here but the confidence limit is shown anyway, in order to provide some indication of what the level uncertainty might be.

The confidence interval calculation used is a well known statistical method and is described and justified by, for example, Hollander and Wolfe (1999). Let n be the sample size for a random sample from a continuous distribution. The principle is that the upper confidence limit is the r th order statistic (r th value in sample sorted in increasing numerical order) where r is the smallest whole number such that the probability that a binomial random variable, with n trials and probability α of success in each trial, produces a value less than r is at least the desired confidence level.

Table B.7: Empirical estimates of percentiles, with upper confidence limits, for three percentiles of variability and four formulation categories

| Formulation category | Dilution/concentrate | Sample size | Quantile 95% | Upper bound 95% CI | Quantile 97.5% | Upper bound 95% CI | Quantile 99% | Upper bound 95% CI |
|----------------------|----------------------|-------------|--------------|--------------------|----------------|--------------------|--------------|--------------------|
| Organic solvent | Concentrate | 1,153 | 18 | 20 | 24 | 30 | 32 | 44 |
| Water based | Concentrate | 1,073 | 8 | 10 | 16 | 21 | 28 | 41 |
| Solid | Concentrate | 471 | 8 | 11 | 11 | 14 | 14 | 17 |
| Other | Concentrate | 131 | 20 | NA | 65 | NA | 84 | NA |
| Organic solvent | Dilution | 1,553 | 49 | 55 | 64 | 67 | 73 | 80 |
| Water based | Dilution | 1,567 | 40 | 44 | 55 | 60 | 69 | 75 |
| Solid | Dilution | 710 | 51 | 57 | 63 | 72 | 74 | 102 |
| Other | Dilution | 105 | 56 | 61 | 58 | NA | 61 | NA |

CI: confidence interval; NA: situations in which there was insufficient data to obtain an upper confidence limit.

Table B.8: Empirical estimates of percentiles, with upper confidence limits, for three percentiles of variability and two formulation categories

| Formulation category | Dilution/concentrate | Sample size | Quantile 95% | Upper bound 95% CI | Quantile 97.5% | Upper bound 95% CI | Quantile 99% | Upper bound 95% CI |
|-------------------------|----------------------|-------------|--------------|--------------------|----------------|--------------------|--------------|--------------------|
| Organic solvent + other | Concentrate | 1,284 | 18 | 20 | 25 | 31 | 35 | 50 |
| Water based + solid | Concentrate | 1,544 | 8 | 9 | 13 | 16 | 24 | 33 |
| Organic solvent + other | Dilution | 1,658 | 51 | 55 | 62 | 67 | 73 | 80 |
| Water based + solid | Dilution | 2,277 | 45 | 48 | 57 | 61 | 70 | 74 |

CI: confidence interval.

5.2. Model based

The random effects logit regression model described in Section 3.2 of Appendix B was used to make calculations to support setting of default values.

The model includes random effects for active substances (and related variables). This means that the effects for active substances are modelled as being sampled from a normal distribution. The parameters of that distribution are among those estimated from the data when fitting the model. The unknown effect for a new substance can be seen as a draw from that distribution and this quantifies uncertainty about absorption for the new substance. The same approach can be taken for each random effect in the model and so it becomes important to decide which sources of variation should be addressed/covered by the default value.

Source block 1 in Tables B.5 and B.6 defines the eight categories described at the start of Section 5 in Appendix B. Source block 2 relates directly to variation between active substances and the default value should address this. Source block 4 (replicate variation) is driven by inter- and intrahuman variability and by intralaboratory variation and therefore need not be addressed by the default value. That leaves source block 3 for which there is a decision to be taken between three possibilities for addressing the variation contained in it: (a) exclude it altogether, (b) include only the between-study variation, (c) include both sources in block 3 (between- and within-study variation).

Having decided which random effects sources should be covered by the calculated values, there are two further decisions to be made. The first is what percentile of the random variation is of interest; if only source block 2 is included in the calculation, this is equivalent to asking what percentage of active substances should be covered by the calculated value. The calculated value can then be determined from the parameter estimates for the random effects model. However, there is uncertainty about the parameters as they are estimated from data and so the second decision is how to address that uncertainty, for example by taking a central estimate or by considering an upper credible limit.

Table B.9 shows calculated values based on the 95th percentile of random effects variation for each of the eight categories. In each category, values are shown for each of options (a), (b) and (c) and uncertainty is addressed by showing the central estimate of the default value and the upper 95th percentile of uncertainty about the default value. Tables B.11 and B.13 show results for the 97.5th and 99th percentiles. Tables B.10, B.12 and B.14 show the corresponding results for four categories.

Table B.9: Values calculated from random effects logit regression model to support setting default values based on the 95th percentile of variability for formulations in four categories

| Formulation category | Dilution/concentrate | Options for sources of variation from source block 3 to be covered by the default value | | | | | |
|----------------------|----------------------|---|-----------|------------------------|-----------|---------|-----------|
| | | (a) None | | (b) Between-study only | | (c) All | |
| Organic solvent | Concentrate | 5 | <i>7</i> | 8 | <i>10</i> | 11 | <i>14</i> |
| Water based | Concentrate | 3 | <i>4</i> | 4 | <i>6</i> | 6 | <i>8</i> |
| Solid | Concentrate | 3 | <i>4</i> | 4 | <i>6</i> | 6 | <i>8</i> |
| Other | Concentrate | 3 | <i>6</i> | 5 | <i>8</i> | 7 | <i>11</i> |
| Organic solvent | Dilution | 37 | <i>45</i> | 48 | <i>56</i> | 57 | <i>64</i> |
| Water based | Dilution | 24 | <i>30</i> | 33 | <i>39</i> | 41 | <i>47</i> |
| Solid | Dilution | 24 | <i>30</i> | 33 | <i>39</i> | 41 | <i>48</i> |
| Other | Dilution | 27 | <i>39</i> | 37 | <i>49</i> | 46 | <i>58</i> |

Upper 95th percentile values are presented in italics.

Table B.10: Values calculated from random effects logit regression model to support setting default values based on the 95th percentile of variability for formulations in two categories

| Formulation category | Dilution/concentrate | Options for sources of variation from source block 3 to be covered by the default value | | | | | |
|-------------------------|----------------------|---|-----------|------------------------|-----------|---------|-----------|
| | | (a) None | | (b) Between-study only | | (c) All | |
| Organic solvent + other | Concentrate | 5 | <i>7</i> | 8 | <i>10</i> | 11 | <i>13</i> |
| Water based + solid | Concentrate | 3 | <i>4</i> | 4 | <i>6</i> | 6 | <i>8</i> |
| Organic solvent + other | Dilution | 36 | <i>44</i> | 47 | <i>54</i> | 56 | <i>62</i> |
| Water based + solid | Dilution | 23 | <i>29</i> | 32 | <i>38</i> | 40 | <i>46</i> |

Upper 95th percentile values are presented in italics.

Table B.11: Values calculated from random effects logit regression model to support setting default values based on the 97.5th percentile of variability for formulations in four categories

| Formulation category | Dilution/concentrate | Options for sources of variation from source block 3 to be covered by the default value | | | | | |
|----------------------|----------------------|---|-----------|------------------------|-----------|---------|-----------|
| | | (a) None | | (b) Between-study only | | (c) All | |
| Organic solvent | Concentrate | 7 | <i>10</i> | 11 | <i>15</i> | 16 | <i>20</i> |
| Water based | Concentrate | 4 | <i>5</i> | 6 | <i>8</i> | 9 | <i>12</i> |
| Solid | Concentrate | 4 | <i>6</i> | 6 | <i>9</i> | 9 | <i>12</i> |
| Other | Concentrate | 4 | <i>7</i> | 7 | <i>12</i> | 11 | <i>17</i> |
| Organic solvent | Dilution | 45 | <i>54</i> | 58 | <i>66</i> | 68 | <i>74</i> |

| Formulation category | Dilution/concentrate | Options for sources of variation from source block 3 to be covered by the default value | | | | | |
|----------------------|----------------------|---|-----------|------------------------|-----------|---------|-----------|
| | | (a) None | | (b) Between-study only | | (c) All | |
| Water based | Dilution | 29 | <i>37</i> | 41 | <i>49</i> | 52 | <i>59</i> |
| Solid | Dilution | 29 | <i>38</i> | 42 | <i>50</i> | 52 | <i>59</i> |
| Other | Dilution | 34 | <i>46</i> | 46 | <i>59</i> | 57 | <i>69</i> |

Upper 95th percentile values are presented in italics.

Table B.12: Values calculated from random effects logit regression model to support setting default values based on the 97.5th percentile of variability for formulations in two categories

| Formulation category | Dilution/concentrate | Options for sources of variation from source block 3 to be covered by the default value | | | | | |
|-------------------------|----------------------|---|-----------|------------------------|-----------|---------|-----------|
| | | (a) None | | (b) Between-study only | | (c) All | |
| Organic solvent + other | Concentrate | 7 | <i>9</i> | 11 | <i>14</i> | 16 | <i>20</i> |
| Water based + solid | Concentrate | 4 | <i>5</i> | 6 | <i>8</i> | 9 | <i>12</i> |
| Organic solvent + other | Dilution | 43 | <i>52</i> | 56 | <i>64</i> | 66 | <i>72</i> |
| Water based + solid | Dilution | 29 | <i>36</i> | 41 | <i>48</i> | 52 | <i>58</i> |

Upper 95th percentile values are presented in italics.

Table B.13: Values calculated from random effects logit regression model to support setting default values based on the 99th percentile of variability for formulations in four categories

| Formulation category | Dilution/concentrate | Options for sources of variation from source block 3 to be covered by the default value | | | | | |
|----------------------|----------------------|---|-----------|------------------------|-----------|---------|-----------|
| | | (a) None | | (b) Between-study only | | (c) All | |
| Organic solvent | Concentrate | 10 | <i>14</i> | 17 | <i>22</i> | 25 | <i>31</i> |
| Water based | Concentrate | 5 | <i>8</i> | 9 | <i>13</i> | 15 | <i>19</i> |
| Solid | Concentrate | 5 | <i>8</i> | 9 | <i>13</i> | 15 | <i>20</i> |
| Other | Concentrate | 6 | <i>10</i> | 11 | <i>17</i> | 17 | <i>26</i> |
| Organic solvent | Dilution | 53 | <i>63</i> | 68 | <i>75</i> | 78 | <i>83</i> |
| Water based | Dilution | 37 | <i>47</i> | 53 | <i>61</i> | 65 | <i>71</i> |
| Solid | Dilution | 37 | <i>47</i> | 53 | <i>61</i> | 65 | <i>72</i> |
| Other | Dilution | 42 | <i>55</i> | 58 | <i>69</i> | 69 | <i>79</i> |

Upper 95th percentile values are presented in italics.

Table B.14: Values calculated from random effects logit regression model to support setting default values based on the 99th percentile of variability for formulations in two categories

| Formulation category | Dilution/concentrate | Options for sources of variation from source block 3 to be covered by the default value | | | | | |
|-------------------------|----------------------|---|-----------|------------------------|-----------|---------|-----------|
| | | (a) None | | (b) Between-study only | | (c) All | |
| Organic solvent + other | Concentrate | 9 | <i>13</i> | 16 | <i>21</i> | 24 | <i>30</i> |
| Water based + solid | Concentrate | 5 | <i>8</i> | 9 | <i>13</i> | 15 | <i>19</i> |
| Organic solvent + other | Dilution | 51 | <i>61</i> | 67 | <i>74</i> | 77 | <i>82</i> |
| Water based + solid | Dilution | 36 | <i>46</i> | 52 | <i>60</i> | 64 | <i>70</i> |

Upper 95th percentile values are presented in italics.

5.3. Advantages and disadvantages of the two different approaches

The empirical approach has the merit that it is easily understood and is relatively easy to implement. However, it has some weaknesses:

- when a particular percentile of variability is chosen, it includes all sources of variability including inter- and intra-human and intra-laboratory which should really be excluded;
- it gives equal weight to each record in the data set and takes no account of the fact that there are more studies (and a wider variety of experimental conditions) for some substances than others, and a varying number of replicates;
- the method used to assess uncertainty about the chosen percentile is designed for a random sample from a homogeneous population rather than for a hierarchically structured data set.

The modelling approach addresses all the weaknesses identified for the empirical approach but has potential weaknesses of its own:

- there is always some doubt about the selection of particular statistical model and no evaluation has been made of alternative models;
- in particular, the model assumes that random effects are homogeneous across the 8 (or 4) categories, whereas the empirical approach treats each category separately;
- the model makes distributional assumptions which may not be valid: random effects and replicate variation are both assumed to be normally distributed.

Some identified weaknesses of the modelling approach could be addressed by further statistical analysis, whereas the weaknesses of the empirical approach are difficult to overcome. Possible enhancements to the statistical model include relaxation of various normal distribution assumptions, introduction of heterogeneity between the categories and the incorporation of additional predictive physicochemical properties.

Both approaches assume that the substances and the formulations in the data set (ECPA + BfR) are fully representative for products and their in-use dilutions to which default values would in future be applied. As the latter cannot be predicted, it was considered technically not feasible to perform a corresponding analysis of representativeness/coverage of the relevant chemical space.

6. Proposals for overall default values

The data were analysed by two different approaches as (see Section 5 in Appendix B):

- 1) The percentage upper confidence level/credibility limit of a percentile of the empirical absorption data;
- 2) Random effects logit regression modelling.

Each approach has their merits and drawbacks as discussed and it was not possible to conclude which performed best.

Therefore, the proposals below are based on an integrating the outcomes of the two approaches and also taking into account the previous default values and the adaptations of the default values reported in the PPR Panel guidance issued in 2012 (EFSA PPR Panel, 2012) are proposed as specified in Table B.15.

Table B.15: Default values to be used in the absence of experimental data

| Formulation category | Concentration status | Default value (%) |
|--|----------------------|-------------------|
| Organic solvent-based ^(a) and others ^(b) | Concentrate | 25 |
| | Dilution | 70 |
| Water-based/dispersed ^(c) or solid ^(d) | Concentrate | 10 |
| | Dilution | 50 |

(a): Emulsifiable concentrate (EC), emulsion, oil in water (EW), suspo-emulsion (SE), dispersible concentrate (DC), oil miscible liquids (OL/OF), oil-based suspension concentrates (OD), emulsion for seed treatment (ES), microemulsion (ME).

(b): Bait concentrate (CB), capsule suspension (CS), gel for direct application (GEL/GD), bait, ready for use (RB), mixture of capsule suspension and suspension concentrate (ZC), seed coated with a pesticide (PS), experimental solution of active substances in solvent (AI).

(c): Soluble concentrate (SL), suspension concentrate (SC), flowable concentrate for seed treatment (FS), flowable (FL) (=SC).

(d): Wettable powder (WP), water-dispersible granules (WG/WDG), water-soluble granules (SG), water-soluble powder (SP), powder for dry seed treatment (DS).

These proposals are pragmatic and represent a reasonable worst case that takes into account the 95% upper confidence/credibility limit of the 95th percentile of the respective empirical relative absorption data for the different formulations and of the relative absorption data predicted using random effects logit regression modelling, as well as the remaining uncertainties relating to the representativeness of the data set (ECPA + BfR). They are specified by formulation category and concentration status ('concentrate' or 'dilution') as the statistical analyses performed on the EFSA pesticide data set (ECPA + BfR) indicated that these parameters were the main drivers of variation in the absorption data.

The default value for diluted organic-based product is proposed to be 70%, constituting an overall value covering the upper confidence/credibility limits of both analyses. This value is also proposed for the dilutions of 'other formulations', since the results obtained from the statistical analyses performed do not justify deviation from the highest default of 70% established in the PPR Panel guidance (EFSA PPR Panel, 2012) in view of the scarcity of the data available for this heterogeneous category.

For solids and water-based products, both concentrated and diluted, default values can be substantially revised based on the new data set (ECPA + BfR). Values of 10% and 50%, for concentrated and diluted product, respectively, are proposed, reflecting the outcomes of both analyses carried out.

The default value for the organic-based formulations is proposed to be maintained at 25% for concentrated products, since the analysis of the new data set does not suggest a substantially different value. With regard to the category 'other formulations', new data were scarce and members of the category very diverse, which was considered no robust basis to suggest a revision of the default value.

It follows that if more data become available, especially for the formulation category 'other', revision of default values and categorisation could be considered.

Appendix C – Evaluation of dermal absorption guidance/guideline documents

1. Data and methodology

The evaluation of available documents on dermal absorption was performed in order to identify potential inconsistencies/discrepancies among them on the same factor/criterion covering both experimental and data analysis aspects. Moreover, identified reference documents were also screened to collect different level of details provided for the same factor/criterion.

Information on the conduction and interpretation of dermal absorption experiments for chemicals described in OECD Test Guideline (TG) 428 (2004b), OECD guidance 28 (2004c) and OECD guidance notes on dermal absorption (2011) were evaluated against EFSA PPR Panel guidance on dermal absorption (EFSA PPR Panel, 2012) developed for PPPs, and the EFSA opinion on the 'Science behind the revision of the guidance document on dermal absorption' (EFSA PPR Panel, 2011). Other general guidance documents on dermal absorption (Hoang, 1992; WHO, 2006; ECETOC, 2013) as well as guidance developed for specific products such as cosmetics (SCCS, 2010, 2015) and medicines (EMA, 2014) were included in the analysis.³⁵ Deviations from OECD and EFSA dermal absorption documents when performing the study were collected during the quality check of new submitted human *in vitro* experiments, described in the EFSA Scientific Report 'Assessment of new scientific studies on human *in vitro* dermal absorption' (EFSA, 2015).

Critical information on the conduction and the assessment of human *in vitro* dermal absorption studies were grouped into categories and evaluated through the documents accordingly:

- i) experimental aspects, such as: study design, skin origin/region/type/preparation, sampling period, washing procedure, tape stripping, etc.; factors possibly influencing dermal absorption, such as humidity, temperature, state of occlusion, skin site, skin irritation, etc.;
- ii) data analysis, such as: recommendations to account for experimental variability, to identify outliers, to calculate $t_{0.5}$, criteria for recovery acceptability, recommended default values (DVs), etc.

2. Results

The evaluation of collected information from the selected dermal absorption documents covering the same factor/criterion evidenced the following deviances/discrepancies:

- i) Experimental variability (intra-assay):
 - EFSA (EFSA PPR Panel, 2012), like SCCS (2010, 2015), recommends the use of eight skin samples from at least four donors for a reliable dermal absorption study. A minimum of four replicates³⁶ per test preparation is requested by OECD TG 428 (2004b). EMA (2014) recommends to use a sufficient number of replicates (the choice should be demonstrated to be statistically significant), typically six or more for pivotal experiments, and at least two different donors.
 - From analysis of submitted study reports, the EFSA/SCCS recommendation to reduce experimental variation, which was based on practical experience with the type of study, is not followed as only the OECD minimum requirement (four replicates, irrespective of the donors) is often applied.
 - No instructions are provided by any of the screened documents on criteria to be applied for the identification of outliers. For both EFSA (EFSA PPR Panel, 2012) and OECD (2011), the reason for excluding outliers should be clearly stated in the study report (and in the summary text for EFSA). Moreover, the full results from samples considered to be outliers must be presented and results treated as outliers should include spuriously low as well as high values. However, from the check of submitted study reports these recommendations are not routinely followed.

³⁵ WHO/ECETOC/EPA documents were screened to collect different level of details provided for the same factor/criterion.

³⁶ EFSA (2012): If results from four replicates are not available, it is possible to use the mean value of three replicates if the results are closely matched or, if there is significant variation, take the highest value rather than the mean.

- EFSA (EFSA PPR Panel, 2012)³⁷ and OECD (2011) approach for high variability within replicates (i.e. standard deviation is equal or larger than 25% of the mean of the absorption as defined in Section 5.6 and 5.8) to add a standard deviation to the mean value is not followed by ECPA (Aggarwal et al., 2014, 2015), as evidenced from the quality check of submitted study reports. For cosmetics (SCCS, 2015), a standard deviation should be added to the mean value by default (two standard deviations in case of deviations from protocol and/or very high variability).
- ii) Data analysis:
- Recovery acceptability criteria: OECD TG 428 (2004b) requires mean mass balance recovery of the test substance between 90% and 110% ($100 \pm 10\%$). The same recommendation is expressed in OECD guidance document 28 (2004c) plus, under certain circumstances, such as for a volatile substance, a range of 80–120% ($100 \pm 20\%$) is acceptable. EFSA (EFSA PPR Panel, 2012) requirement for recovery is 95–105%, whereas the SCCS (2015) criterion is less stringent (85–115%). The EFSA range for acceptable recovery (including normalisation when recovery is outside the range) is not applied by ECPA (Aggarwal et al., 2014, 2015) and from the evaluation of submitted studies; it was that 96.4% records met the OECD criterion. EMA guidance (2014) provides the same recommendation as OECD TG 428 (2004b).
 - Default values: OECD (2011) and EFSA (EFSA PPR Panel, 2011, 2012) both recommend to use, in the absence of experimental data, a default of 10% for substances with $MW > 500$ and $\log P_{ow} < -1$ or > 4 . Additional default values of 25% and 75% are recommended by EFSA³⁸ (2011; 2012) for products containing $> 5\%$ active substance and for products or in-use dilutions containing $\leq 5\%$ active substance, respectively. OECD (2011) assumes that as a first step, a default value of 100% should be used in exposure calculation, to cover a worst-case scenario. For cosmetics (SCCS, 2015), default values of 10% (as OECD) and 50% are recommended.
 - t0.5 calculation: EFSA's (EFSA PPR Panel, 2011, 2012) recommendation for the exclusion of tape strips from the dermal absorption calculation is based on the calculation of t0.5, i.e. the 'total absorption (material in the receptor fluid at the end of the study) occurred within half duration of the total sampling period'. From evaluation of submitted study reports, it was found that, based on EFSA's definition of t0.5, at least three different approaches were applied to calculate t0.5, leading to different results. The same approach is recommended by the OECD guidance notes 156 (2011). However, none of the documents provides detailed guidance on the calculation of the value, which in practice has resulted in diverging assessments of the same data. Also, this approach has been criticised for not making adequate use of the data generated at many different time points as it is required by the OECD TG 428 (2004b).
 - Pro-rata/Bridging: EFSA (EFSA PPR Panel, 2011, 2012) recommends a 'pro rata approach' to extrapolating dermal absorption values from tested dilutions to non-tested dilutions of the same concentrate (for PPPs). For bridging of studies on dermal absorption performed with tested formulations to non-tested formulations, EFSA (EFSA PPR Panel, 2012) defines criteria for similarity of formulations.

3. Conclusions

The evaluation of existing documents on dermal absorption has identified diverging recommendations to reduce experimental variability, recovery acceptability criteria and default values to be used in the absence of experimental data. This analysis clearly indicates the need for improved harmonisation among guidance/guideline documents on dermal absorption. In addition, from the quality check of the submitted study reports, it was noted that some recommendations are not routinely followed when performing, analysing and reporting studies. In some cases, even when an aspect is covered in a single document, the need for clarifying the recommendations was identified (t0.5 calculation, now clarified in Section 5.1). Although studies are, in general, in line with requirements in OECD TG 428 (OECD 2004b), this document provides only few details on experimental

³⁷ A revised approach for the treatment of variability within the results is provided in the current guidance (see Section 5.3).

³⁸ New default values are reported in the current guidance, revised on the basis of the analysis of new human *in vitro* dermal absorption data (see Section 6.1, flow chart 1a and Appendix B).

conduct, and data analysis and reporting recommendations are not included, as covered in guidance documents (OECD, 2011; EFSA PPR Panel, 2012).

In the absence of data, several approaches are currently used by different competent authorities as well as different applicants. Thus, defined rules are needed to harmonise mutual regulatory acceptance of assessments generated across different regulatory purposes.

It is therefore recommended to revise the OECD dermal absorption guidance, guidance document and test guideline documents in order to support a more harmonised approach for the evaluation of dermal absorption of chemicals, reflect more appropriately the extensive experience gained using the *in vitro* methods, and to increase global acceptance of this method as a replacement for testing in animals.

Appendix D – Evaluation of literature on QSAR for skin absorption prediction

1. QSARs

Typically, QSARs are based on training sets derived from *in vitro* dermal absorption experiments. Most QSARs developed to predict skin permeation of chemicals target the permeation constant (k_p), and a few maximum flux (J_{max}) (Mitragotri et al., 2011). Occasionally, also QSARs for percentage absorption have been developed (e.g. by Gute et al., 1999), but although preferred from a regulatory point of view, they have limited value, since this parameter is highly influenced by exposure conditions such as duration and dermal loading.

Many algorithms of global QSARs for aqueous solutions have been reviewed in the publically available literature (Buist, 2016). The majority of these QSARs relate permeation to the octanol–water partition coefficient (K_{OW}) and molecular weight (MW). When tested, log K_{OW} and MW-based QSARs did not provide good predictions with external validation sets (R^2 for the correlation between observed and predicted values <0.5). Also, QSARs using molecular descriptors other than log K_{OW} and MW did not perform well with external validation sets. A possible explanation for the bad performance of these QSARs may be that they are mainly derived by multiple linear regression, whereas the relation between k_p or J_{max} and at least some of the descriptors used is not always linear.

New developments in QSAR development include neural network, nearest neighbour and Gaussian process models (Buist, 2016). These novel models predict non-linear processes, and Gaussian process models have been shown to perform better than selected QSARs, as have fuzzy models and artificial neural networks. However, they yet have to prove their practical value, e.g. when tested with external validation sets.

Little work has been done to include vehicle effects in QSAR predictions, most of which include a mixture factor, a composite descriptor (Buist, 2016). Each of these QSARs were based on a small training set of less than 20 molecules, and all showed quite different mixture factors.

2. Mathematical skin models

The QSARs described in the previous section merely predict a constant (k_p or J_{max}) of the skin permeation process. In order to calculate actual amounts of permeant crossing the skin under relevant exposure conditions, a mathematical skin model is needed. The simplest model only considers the *stratum corneum*, being the major barrier for dermal absorption in most cases, and regards it as homogeneous compartment (see Russell and Guy, 2009; Mitragotri et al., 2011; Anissimov et al., 2013). Also, more complex models of the skin mainly concern the *stratum corneum*. Most of them describe the structure of the *stratum corneum* more accurately as a so called 'brick-and-mortar' model (Mitragotri et al., 2011; Anissimov et al., 2013). Although receiving less attention, viable epidermis and avascular and vascular dermis have been modelled as well (Anissimov et al., 2013). Every compartment added to the skin model implies an increase in parameters to describe partitioning between the different compartments and diffusion across them, and increases the mathematical complexity of the models and thereby the data needs of the models in order to determine the various chemical-specific and skin specific parameters (Mitragotri et al., 2011; Anissimov et al., 2013). This means that, in general, the usability of the more complex models in regulatory risk assessment will be limited, due to lack of data to populate the models with the necessary parameters. Furthermore, specialist numerical skills are required to use complex models (Anissimov et al., 2013). The latter objection is mitigated by a versatile and complex skin model, the Finite Dose Skin Permeation Model (FDSP) made available on the Internet³⁹ by the US Centers for Disease Control and Prevention (CDC). Unfortunately, it did not perform well with an external validation data set (Buist, 2016).

Mathematical skin models need the input chemical-specific parameters like k_p , however, as explained above, no reliable method to predict k_p is currently available (Buist et al., 2010; Buist, 2016).

3. Recommendations

In future, the current QSARs for k_p could be replaced by models with better predictive qualities to be used with selected mathematical skin models to predict dermal absorption, as defined in this guidance (as the sum of the relative amount permeated into the receptor fluid and the relative amount

³⁹ <http://www.cdc.gov/niosh/topics/skin/finiteSkinPermCalc.html>

still present in the skin (minus the first two tape strips)). Besides $\log K_{OW}$ and MW, the physicochemical parameters that are used in most of the dermal absorption QSARs, an additional predictive independent variable is needed which describes the chemical reactivity of the substance, e.g. hydrogen bonding. Furthermore, the k_p model to be developed should be non-linear with respect to the relationships between k_p and the predictive independent variables, since non-linear models seem to outperform the traditionally developed linear models. A disadvantage of non-linear models is that more data are needed to develop them than for linear models, and that they are more prone to overfitting. Therefore, existing dermal absorption data sets, both public and private, could be combined, curated and filtered to build a large homogeneous and consistent data set that can be used to develop such non-linear models to predict k_p .

Concerning the available models for the estimation of dermal absorption of chemicals, including mixtures, briefly reviewed in Sections 1 and 2 of Appendix D, it is recommended to evaluate their applicability to a large and homogenous data set of human *in vitro* dermal absorption studies with PPPs, as the data set (ECPA + BfR) described in Appendix A. An extensive review of all the available models including a critical appraisal based on OECD criteria (OECD, 2007) should be conducted to select models to be used for testing their predictability and reliability of predictions of dermal absorption for pesticides.